THE SUSCEPTIBILITY OF SEED AND SEEDLINGS OF SOME EUCALYPTUS AND PINUS SPP. TO A SELECTION OF SOIL-BORNE PATHOGENS

by

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ABSTRACT

A range of *Phytophthora*, *Pythium* and *Fusarium* spp., some of which were isolated from native vegetation, were tested for pathogenicity on a selection of *Eucalyptus* and *Pinus* spp.

Initially the interaction between fungi and seedlings was studied using a system in which the biological factors were minimized. The introduced fungus faced few if any antagonists and the host could grow in a soil environment relatively free from other stimulatory or inhibiting micro-organisms. Isolates of *Ph. cinnamomi*, *Ph. drechsleri*, *P. ?acanthicum*, *P. ?deliense*, *P. myriotylum* and *P. splendens* were found to cause pre- and post-emergence damping-off in many of the *Eucalyptus* and *Pinus* spp. used.

The interaction between a range of the fungi and a selection of the tree species was then studied in unsterilized soils. In two of the soils, a *Phytophthora* sp. was present as a component of the natural soil microflora, no *Phytophthora* spp. could be isolated from the other three soils. To each soil a single pathogen was introduced and the interaction of soil, fungus and host studied. Although, the natural soil microflora was found to influence the pre- and post-emergence damping-off caused by some fungi, for the majority of fungi no effect was evident.

Species resistance to attack by fungal organisms was extremely variable. All species were susceptible to some degree to some fungi but *Eucalyptus maculata* was the only species to consistently demonstrate some resistance to fungal attack.

Of the species of Phytophthora and Pythium frequently recovered from forest soils, some isolates of Ph. drechsleri could be rated as being comparable in pathogenicity with Ph. cinnamomi.

STATEMENT

This dissertation has not previously been submitted for a degree at this or any other University, and is the original work of the writer except where due reference is made in the text.

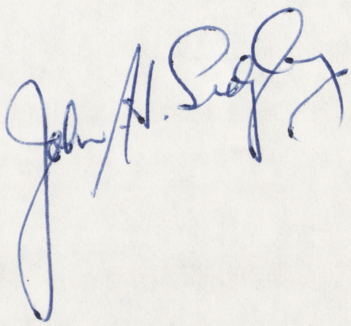

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

DIAGRAM

CHAPTER 1

INTRODUCTION

Many species of the family Myrtaceae are important components of the overstorey and understorey of native forests and woodlands of Australia. Species of the genus *Eucalyptus*, which dominate the overstorey of these associations, are the major local source of hardwood timber.

In recent years the association of soil-borne pathogens with both declining and healthy vegetation in Australia has received considerable attention. *Phytophthora cinnamomi* Rands has been associated with the dieback of *Eucalyptus marginata* Sm. (jarrah) in Western Australia (Podger, Doepel and Zentmyer, 1965; Podger, 1972), mixed *Eucalyptus* spp. in Victoria (Weste and Taylor, 1971; Marks, Kassaby and Reynolds, 1972; Weste, 1974), mixed *Eucalyptus* spp. and understorey plants in New South Wales (Fraser, 1956; Pratt and Heather, 1973a; 1973b; Pratt, Heather and Shepherd, 1973), native vegetation in the Australian Capital Territory (Pratt, Heather, Sedgley and Shepherd, 1972), native vegetation in Queensland (Pratt, Heather, Bolland and Brown, 1972), and native vegetation in Tasmania (Pratt and Heather, 1973a; 1973b). A number of other *Phytophthora* and *Pythium* spp. have also been isolated from samples of soil and plant roots from healthy and declining vegetation in native forests (Pratt and Heather, 1973b; Marks and Kassaby, 1974).

In forest soils losses due to pre-emergence damping-off (Cunningham, 1960) and to the death of seedlings by post-emergence damping-off or root rot normally go unnoticed. Hendrix and Campbell (1973) have suggested that Pythium spp., through the selective elimination of susceptible plant species, may act as potent determinants of forest and plant vegetational associations. A similar role might also be played by species of the related genus Phytophthora.

Phytophthora, Pythium and Fusarium spp. are also recognised as regular inhabitants of forest nursery soils (Vaartaja, Cram and Morgan, 1961; Vaartaja and Salisbury, 1961; Hodges, 1962; Oxenham and Winks, 1963a; 1963b; Vaartaja and Bumbieris, 1964; Vaartaja, 1967; Hendrix and Campbell, 1968; Edmonds and Heather, 1973). However, Fusarium spp. are not recorded as being common components of undisturbed forest soils (Thornton, 1960; Park, 1963; Smith, 1967).

The occurrence of species from these fungal genera, both in the nurseries which supply the stock for extensive reforestation and in the native forest, suggested that there was a need to examine their pathogenicity.

Isolation of fungi, reported in the literature to be potential pathogens, from forest and nursery soils is inadequate on its own. Pathogenicity needs to be proven in the local situation. Pathogenicity is usually established by constant association and isolation of a pathogen, reproduction of the disease symptoms by inoculation and re-isolation of the organism. The pathogenicity so demonstrated is a rather abstract concept for it has not taken into account environmental variables, particularly important in soil-borne diseases.

The initial assessment of pathogenicity is often under experimental conditions that exclude many normal environmental stresses and also components of the biotic environment that may be of supreme importance. The pathogenicity so demonstrated indicates the genetic ability of the species to attack the host: it does not necessarily indicate that attack in field environments will occur.

In this study the pathogenicity of a range of Phytophthora, Pythium and Fusarium spp.* on some Eucalyptus and Pinus spp. was tested:

1. when the host was grown in steam-air-treated sand and hence with limited microbial antagonism to the fungus being studied.
2. in unsterilised soil in the absence of Phytophthora spp. as a component of the natural soil microflora.
3. in soil in the presence of the soil-borne pathogens Phytophthora cinnamomi and Phytophthora drechsleri Tucker.

Finally, the role of a number of these organisms in native vegetation is examined in the light of recent pathological investigations in forestry within Australia.

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* The generic names of the fungal organisms have been abbreviated in the text: F., Fusarium; Ph., Phytophthora; P., Pythium; R., Rhizopus.

CHAPTER 2

THE PATHOGENICITY OF A RANGE OF SOIL-BORNE PLANT FUNGI

2.1 INTRODUCTION

Between January 1969 and August 1972 a survey was undertaken to establish the distribution of Ph. cinnamomi throughout Australia (Pratt and Heather, 1973a). In addition to Ph. cinnamomi, isolations of a number of other Phytophthora and Pythium spp. were made (Pratt and Heather, 1973b). Experiments 1 and 2 are tests to determine the pathogenicity of these species, together with a number of other isolates from agricultural and horticultural plants (Tables 2.1 and 2.2).

'Potential' pathogenicity studies on plants grown in sterilised agar media have been carried out (Vaartaja and Cram, 1956; Vaartaja, Cram and Morgan, 1961; Vaartaja and Salisbury, 1961; Vaartaja and Bumbieris, 1964; Edmonds and Heather, 1973). The pathogenicity so demonstrated is not necessarily indicative of that which occurs in forest environments.

In the following investigation the pathogenicity of a number of organisms, using steam-air-treated sand inoculated with the test fungus, was examined.

2.2 MATERIALS AND METHODS

2.2.1 Apparatus and Experimental Design

The pathogenicity of each fungus was tested on 12 plant species (Diag. 1). Large plastic trays, 8 cm. in depth were used

TABLE 2.1 Fungal Species (*Phytophthora* spp.)

SPECIES	SOURCE
Phytophthora cinnamomi Rands A1	native forest; Ourimbah, N.S.W. IMI 165642.
Phytophthora cinnamomi Rands A2	native forest; W.A.
Phytophthora cactorum (Leb. & Cohn) Schroet.	- - ; W.A.
Phytophthora citricola Sawada	native forest; Ourimbah, N.S.W. IMI 168072.
Phytophthora cryptogea Pethybr. & Laff. A2	horticultural plants; Adelaide, S.A.
Phytophthora cryptogea Pethybr. & Laff. A2	horticultural plants; Adelaide, S.A.
Phytophthora drechsleri Tucker (Northern)*	native vegetation; Cooroy, Queensland. IMI 168071.
Phytophthora drechsleri Tucker A1*	native vegetation; Wilsons Valley, N.S.W. IMI 172308.
Phytophthora drechsleri Tucker A2 (Southern)*	native forest; Condor Creek, A.C.T. K93.
Phytophthora drechsleri Tucker A2 (Southern)*	native forest; Backhouse Creek, N.S.W. K24.
Phytophthora drechsleri Tucker	horticultural plants; Canberra, A.C.T. J8.
Phytophthora drechsleri Tucker	- ; W.A. IMI 129907.
Phytophthora nicotianae Breda de Haan var. *parasitica* (Dastur) Waterh. A1	native forest; Coffs Harbour, N.S.W. IMI 174447.
Phytophthora nicotianae Breda de Haan var. *parasitica* (Dastur) Waterh. A2	agricultural crop; Sandy Creek, near Bermagui-Tathra, N.S.W. IMI 168069.
Phytophthora nicotianae Breda de Haan var. *parasitica* (Dastur) Waterh. A2	native forest; Coffs Harbour, N.S.W. IMI 168070.

TABLE 2.2 Fungal Species (Other than *Phytophthora* spp.)

SPECIES	SOURCE
Fusarium moniliforme var. *subglutinans* Edwards	horticultural plants; Australian National University, Canberra, A.C.T.
Fusarium oxysporum Schlect.	horticultural plants; Australian National University, Canberra, A.C.T.
Pythium (?) *acanthicum* Drechsler*	native forest; Perth Area, Tasmania. IMI 151836.
Pythium (?) *acanthophoron* Sideris*	native forest; Southern Division, Tasmanian Forestry Commission. IMI 151833.
Pythium (?) *deliense* Muers*	native forest; Southern Division, Tasmanian Forestry Commission. IMI 151839.
Pythium irregulare Buisman*	horticultural plants; Canberra, A.C.T. IMI 151841.
Pythium middletonii Sparrow*	- ; Bega, N.S.W. IMI 151834.
Pythium myriotylum Drechsler	agricultural crop; N.S.W.
Pythium (?) *oedochilum* Drechsler*	native vegetation; Wesley Vale, Tasmania. IMI 168074.
Pythium splendens Braun*	native forest; Southern Division, Tasmanian Forestry Commission. IMI 168068.
Pythium ultimum var. *sporangiferum* Drechsler*	native forest; Southern Division, Tasmanian Forestry Commission. IMI 151832.
Pythium sp.	horticultural plants; Canberra. A.C.T. J7.
Pythium sp.	River Sand; Tharwa, A.C.T. J9.
Rhizopus oligosporus Saito	NRRL. 2710. United States.

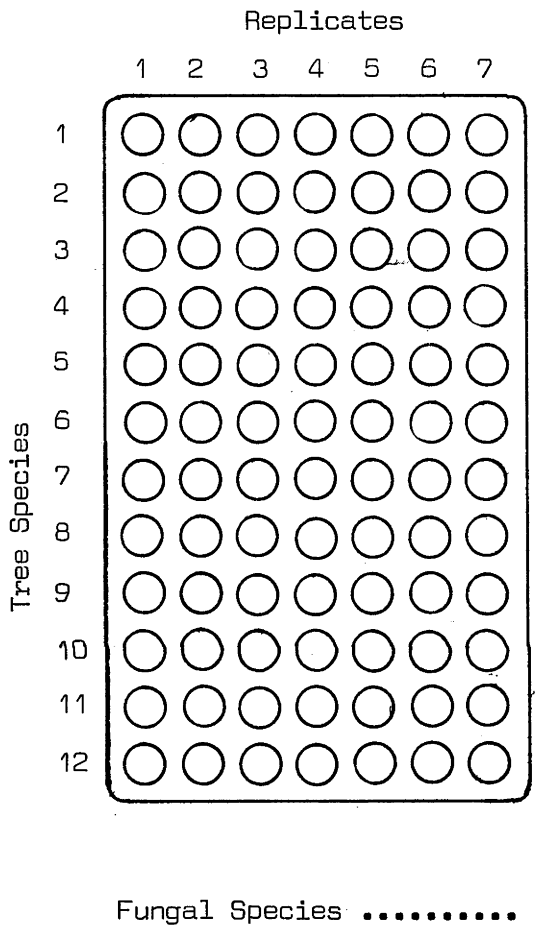

DIAGRAM 1 Pathogenicity Trials; Experimental Layout for Experiments 1 and 2.*

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* Experiments 1 and 2 are similar, each involving the same host species but a different range of fungi. For each Experiment there is a separate control series.

for each test fungus. Plastic drinking cups, 6 cm. high x 5.5 cm. in diameter, with three small holes drilled in the base were used to contain the soil and seeds for each plant species. Seven replicate cups were allowed for each tree species.

2.2.2 The Soil

A washed, coarse-grained river sand was obtained from Tharwa, near Canberra, in the Australian Capital Territory, and treated at 160°C. for 45 min.

Direct soil plating of the sand, after partial sterilization, onto 2% water streptomycin (50 ppm.) agar revealed the absence of fungi but the presence of some bacteria.

2.2.3 The Fungal Species

The isolates used in Experiments 1 and 2 are listed in Tables 2.1 and 2.2. The majority of organisms* used were isolated from native forests by Pratt and Heather (1973b), others were obtained from sources shown.

R. oligosporus was included for comparison of an innocuous organism with suspected pathogens.

2.2.4 The Tree Species

The seed of all species used was collected and cleaned by the Forest Research Institute, Department of Agriculture, Canberra, Australian Capital Territory (Table 2.3).

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* Refer to Tables 2.1 and 2.2.

TABLE 2.3 Seed Sources

Tree	Seed Lot	Location	Viability No Seeds/100 gms.	Weight of (Seed & Frass)/Cup
EUCALYPTUS SPECIES AND SUBGENUS*				
E. cypellocarpa L. Johnson (S.)	9534	Fitzroy Falls, N.S.W.	26,950	0.2 gm.
E. grandis W. Hill ex Maiden (S.)	9683	-	12,600	0.04 gm.
E. maculata Hook (C.)	10611	Woolgoolga, N.S.W.	5,250	0.2 gm.
E. marginata Donn. ex Sm. (M.)	9702	Harvey, W.A.	2.750	0.4 gm.
E. pilularis Sm. (M.)	9457	Nambucca, N.S.W.	7,000	0.4 gm.
E. regnans F. Muell. (M.)	9586	Pennys Saddle, Vic.	13,300	0.1 gm.
E. sieberi L. Johnson. (M.)	9983	Fingal, Tasmania	13,000	0.2 gm.
E. viminalis Labill. (S.)	8899/3	Cann River Area, Vic.	32,900	0.04 gm.
*C., Corymbia; M., Monocalyptus; S., Symphyomyrtus				
*PINUS SPECIES				
P. caribaea Morelet.	10288	South African Plantations	3,150	
P. elliottii Engelm.	8629	Queensland	2,750	
P. pinaster Ait.	9560	Portugal	1,100	
P. radiata D. Don.	10614		2,050	
*Pinus spp. 20 Seeds/Cup.				

2.2.4.1 *Eucalyptus* spp.

Pratt and Heather (1973a) have grouped a number of *Eucalyptus* spp. into four classes according to their field resistance to the disease associated with *Ph. cinnamomi*. In the present study *Eucalyptus* spp. were selected to include representatives of each of the four classes, and to include species of economic importance to the timber industry.

The weight of seed in each cup was selected to provide at least 20 viable seeds.

2.2.4.2 *Pinus* spp.

Pinus spp. were also selected according to their economic importance. They included the majority of species that form the basis of the Softwood reforestation programmes in Australia.

Because of the high viability of seed and ease of counting large seed, 20 seeds were placed in each cup.

2.2.5 Preparation of Inoculated Soil

Inoculum was prepared by growing the test fungus in exfoliated vermiculite moistened with 'Campbells V8' vegetable juice for 4–5 weeks, at 25^{o}C., in the dark. The medium was prepared by modifying the technique of Chilvers (1962) as follows:

To 30 gm. of exfoliated vermiculite was added 200 ml. of diluted V8 juice (150 ml. V8 concentrate to 1 l. of glass distilled water). The moistened vermiculite was sealed in a 500 ml. flask and autoclaved at 240^{o}C. and a pressure of 1.06 kgm./cm^{2}., for 15 min.

When the fungus had spread through the vermiculite, the inoculum was washed with sterile glass distilled water to remove excess nutrients.

The washed inoculum was added to the sand so that the ratio $\frac{\text{DRY WEIGHT OF INOCULUM}}{\text{DRY WEIGHT OF INOCULUM + SAND}}$ was 1:50. Sand and inoculum were mixed by hand for 5 min. Cups were filled to a depth of 4 cm. Sand mixed with washed non-inoculated vermiculite-V8 medium was added to the control cups.

2.2.6 Experimental Procedure

The sand was inoculated by addition of the vermiculite culture and added to the cups (Section 2.2.5).

Weighed seed lots of Eucalyptus spp. or counted seed lots of Pinus spp. were added to the cups and arranged in the trays, so that each of twelve tree species was replicated seven times in each tray. Each tray contained only one test fungus or no added organism in the case of the controls. To each cup was added sufficient steam-air-treated sand to cover the seed.

Watering was carried out by flooding the trays to a depth of 2 cm. with glass distilled water. The trays were then allowed to dry out, then again flooded. This procedure was followed for the duration of the experiment.

Glasshouse conditions were adjusted to provide a 15 h. day with diurnal temperatures varying between 18°C. and 34°C.

The water in each tray was periodically baited, using the technique described by Pratt and Heather (1972), to ascertain the presence of the test organisms.

Observations were made daily. Seedling emergence was assessed at 6–10 days: deaths were also recorded, and the dead seedlings removed. The experiment was terminated at 6 weeks, and the number of seedlings remaining in each cup was recorded.

The roots of live seedlings were plated on 2% water streptomycin (50 ppm) agar, in an attempt to recover the fungus used in the original inoculation.

2.2.7 Statistical Analysis

In both Experiments 1 and 2 the data were analysed in four parts.

1. *Eucalyptus* spp.
   (a) Number of Seedlings Emerged. Any reduction in the number of seedlings emerged, in comparison to the control, was attributed to pre-emergence damping-off.
   (b) Proportion of Seedlings Killed. The death of seedlings emerged was attributed to post-emergence damping-off (No evidence of loss from any other cause was found). Proportion of Seedlings Killed was the ratio

$$\frac{\text{Number of Seedlings Killed}}{\text{Number of Seedlings Emerged}}$$

2. *Pinus* spp.
   (a) Proportion of Seedlings Emerged. This variable was applicable to the *Pinus* spp., since the initial number of sown seeds is known. Proportion of Seedlings Emerged was the ratio

$$\frac{\text{Number of Seedlings Emerged}}{\text{Number of Seeds Sown (20)}}$$

(b) Proportion of Seedlings Killed. As for 1(b).

Transformations of the data were necessary to achieve homogeneity of cell variances. Data for 'Proportion of Seedlings Killed', 1(b) and 2(b), and 'Proportion of Seedlings Emerged', 2(a), were transformed using the arcsine transformation -

$$\sin.^{-1}\sqrt{\frac{\text{Number of Seedlings Emerged (Number Killed)} + .375}{\text{Total Number of Seedlings} + .75}}$$

For 'Number of Seedlings Emerged', 1(a), data were transformed using a square root transformation.

The transformed results were subjected to Analysis of Variance. A 2 factor Analysis of Variance was carried out on the data for 'Number of Seedlings Emerged', 1(a), and 'Proportion of Seedlings Emerged', 2(a), the intracell mean square being used to test for the interaction. The intracell mean square may possibly be an underestimate of the mean square, but in this case it would appear to be a reasonable estimate (Forrester, pers. comm.).

For 'Proportion of Seedlings Killed', 1(b) and 2(b), a 2 factor factorial analysis was again used, but it was non-orthogonal since there were unequal numbers in the sub-classes.

The original data for Experiments 1 and 2 are given in Appendices 1 and 2.

2.3 RESULTS

2.3.1 Pre-Emergence Damping-Off

Statistical Analysis of the data (Table 2.4), supports the conclusion that most of the fungal organisms used were pathogenic

TABLE 2.4 Analysis of Variance for Number of *Eucalyptus* Seedlings Emerged. Experiments 1 and 2

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio (1)	Variance Ratio (2)
Experiment 1				
Fungi	11	94.34		83.95 ***
Trees	7	36.61		32.93 ***
Fungi x Trees	77	5.78	11.83 ***	
Error (Within Sub-class	576	.49		
Error (Interaction +	653	1.11		
Error (Within Sub-class				
Experiment 2				
Fungi	16	153.82		169.89 ***
Trees	7	63.04		69.63 ***
Fungi x Trees	112	5.12	15.68 ***	
Effor (Within Sub-class	816	.33		
Effor (Interaction +	928	.91		
Effor (Within Sub-class				

*** Significant at the 0.1% level

(1) Variance Ratio. Interaction/Within Sub-class

(2) Variance Ratio. Main Effects/Interaction + Within Sub-class

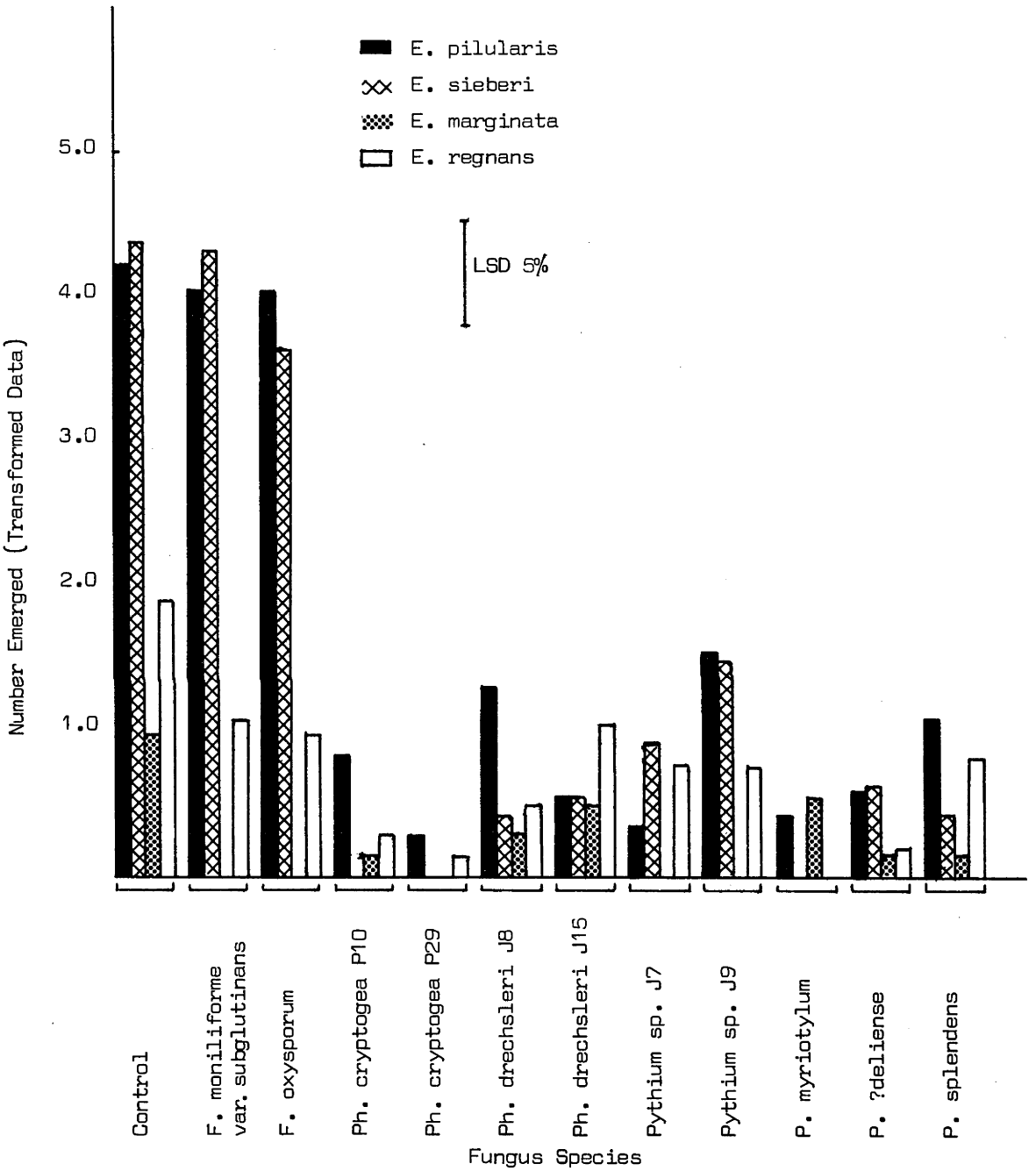

FIG. 2.1 Number of Eucalypt Seedlings Emerged.
Experiment No. 1

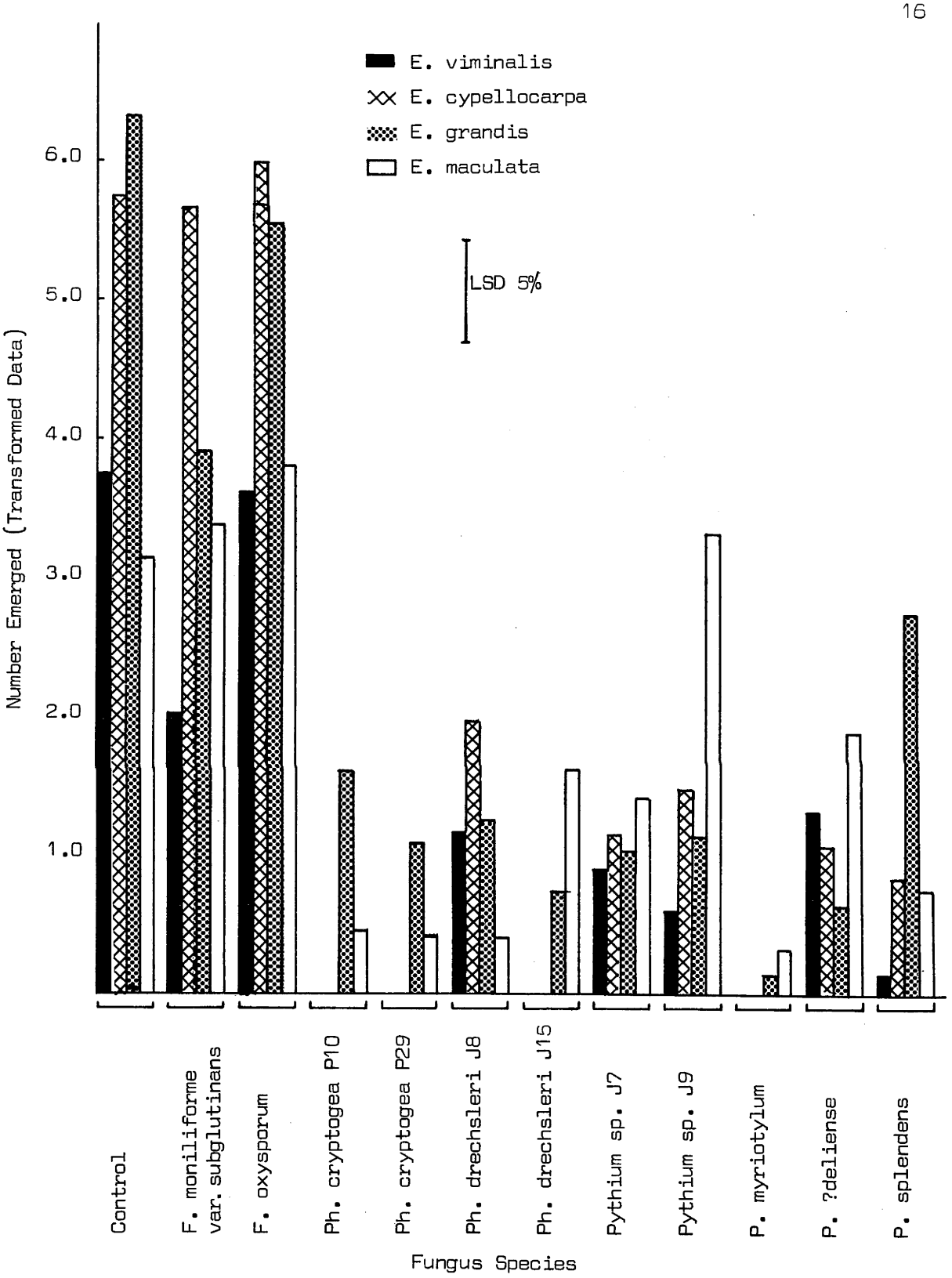

FIG. 2.2 Number of Eucalypt Seedlings Emerged.
Experiment No. 1

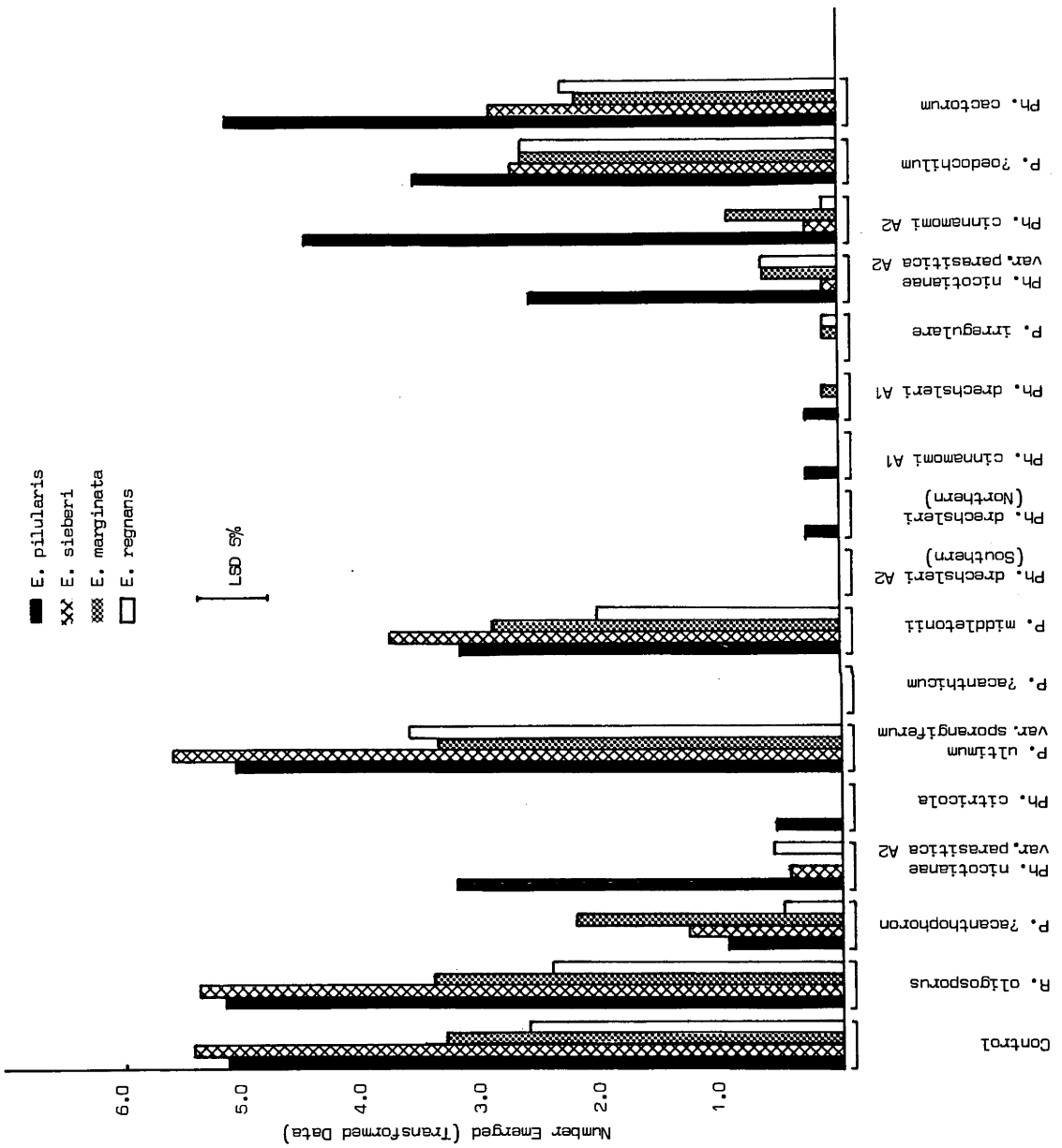

FIG. 2.3 Number of Eucalypt Seedlings Emerged. Experiment No. 2

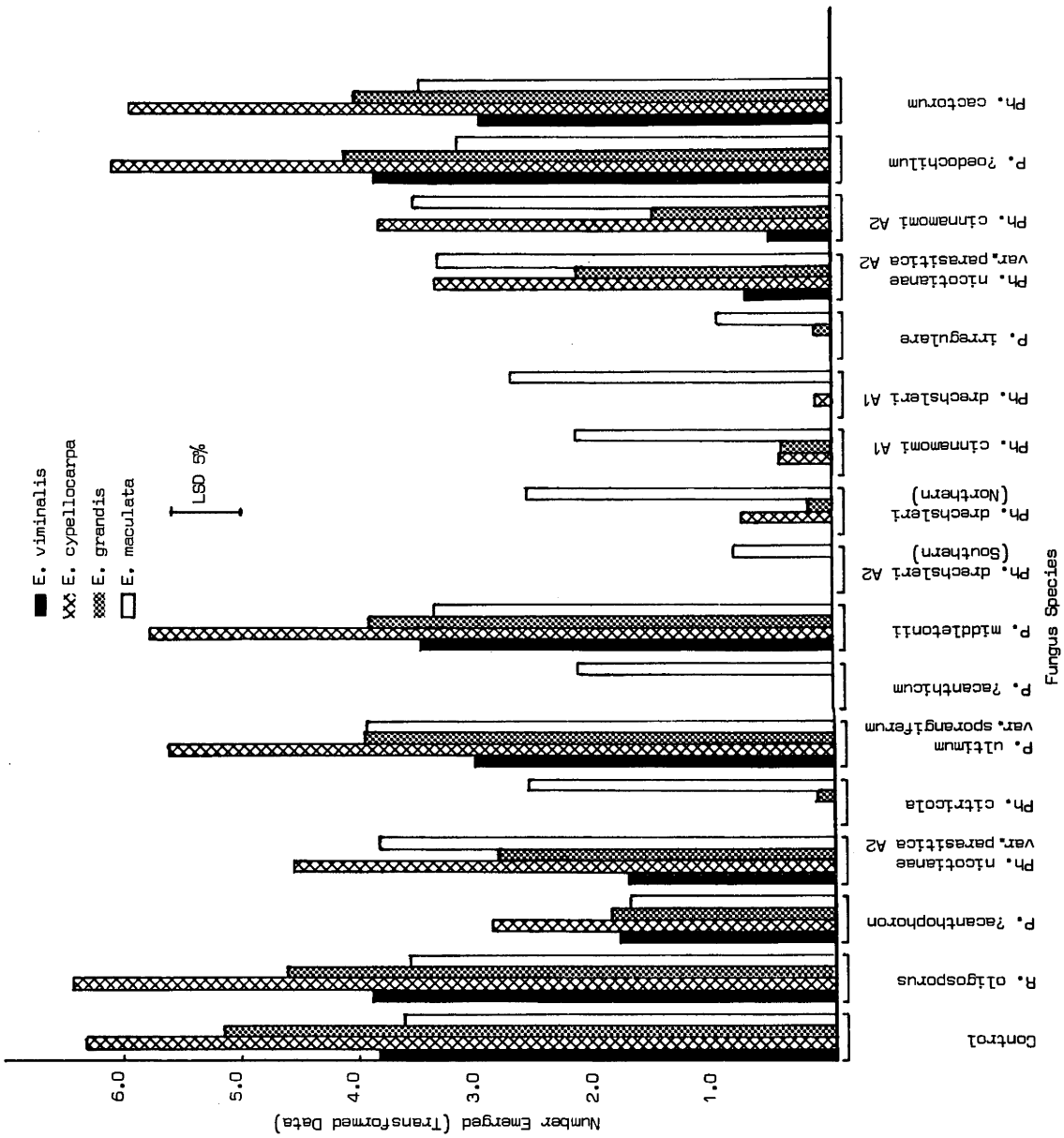

FIG. 2.4 Number of Eucalypt Seedlings Emerged. Experiment No. 2

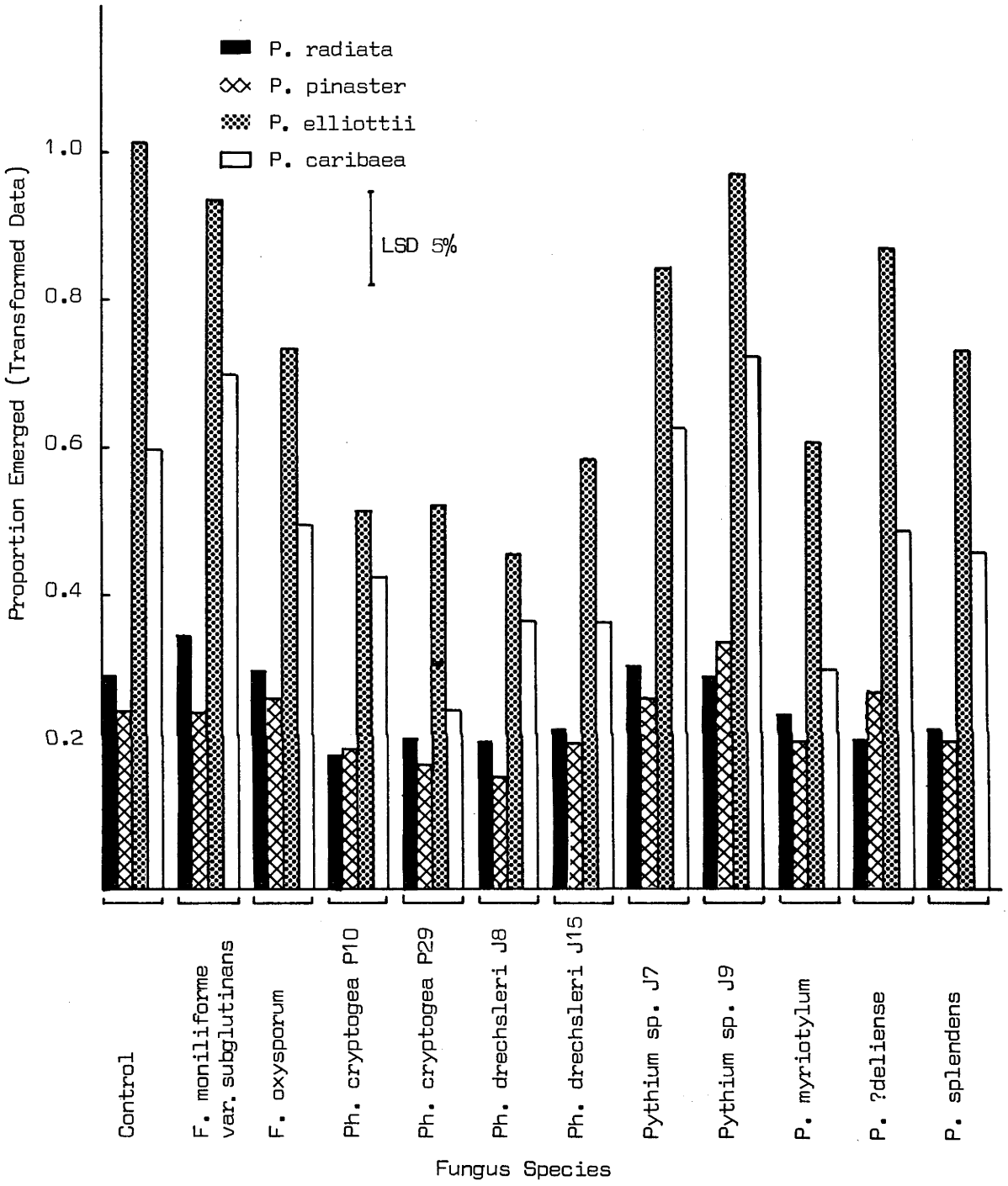

FIG. 2.5 Proportion of Pinus Seedlings Emerged.
Experiment No. 1

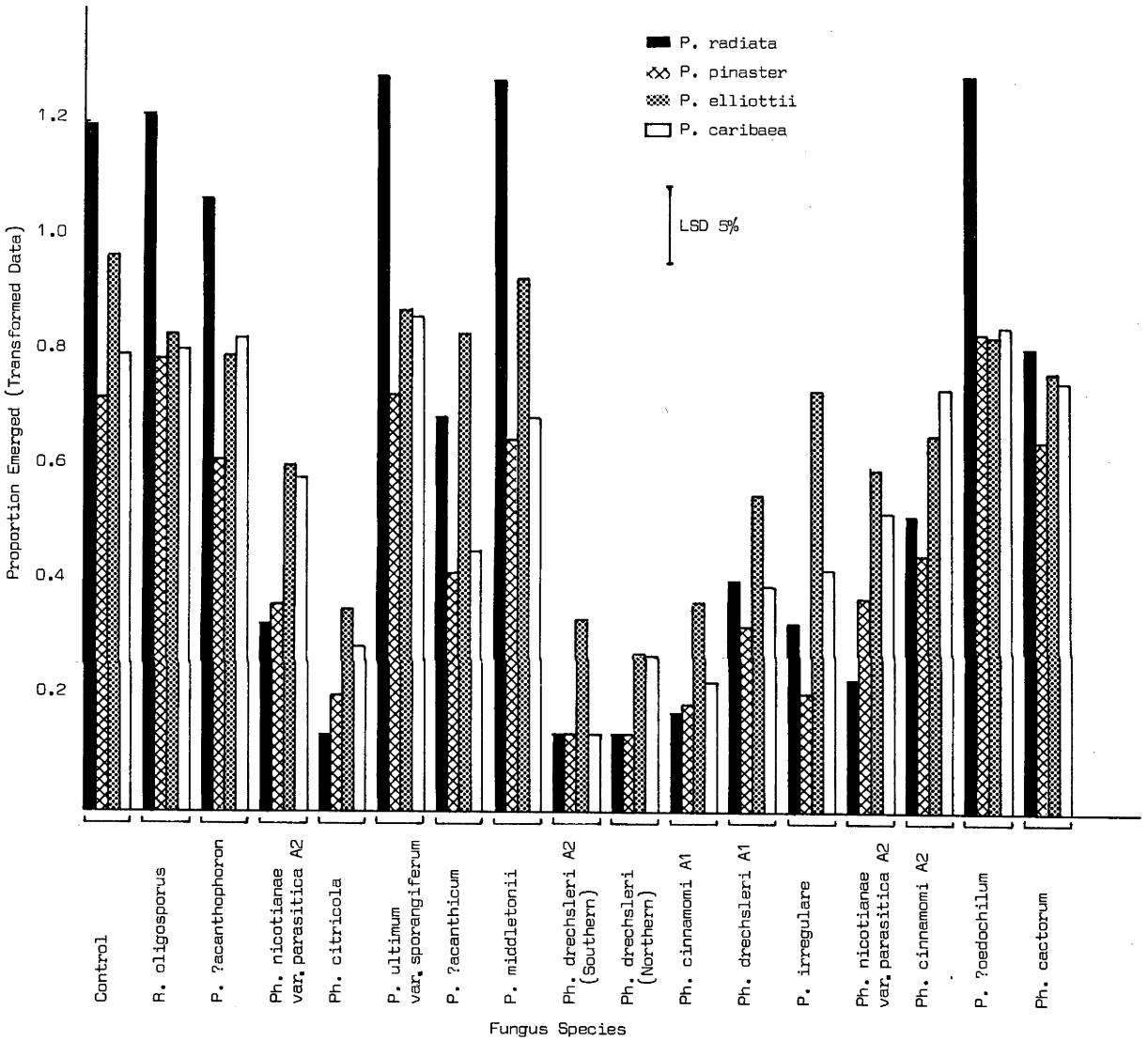

FIG. 2.6 Proportion of Pinus Seedlings Emerged. Experiment No. 2

TABLE 2.5 Summary of Pre-Emergence Damping-Off, Graphically Shown in Figs. 2.1 - 2.6. Comparisons Made Within a Tree Species Using LSD 5%

Fungal Species	Ph. cinnamomi A1	Ph. cinnamomi A2	Ph. cactorum	Ph. citricola	Ph. cryptogea P10	Ph. cryptogea P29	Ph. drechsleri Nth.	Ph. drechsleri A1	Ph. drechsleri K93	Ph. drechsleri J8	Ph. drechsleri J15	Ph. nicotianae var. parasitica A2 J18	Ph. nicotianae var. parasitica A2 J28	F. moniliforme var. subglutinans	F. oxysporum	P. ?acanthicum	P. ?acanthophoron	P. ?deliense	P. irregulare	P. middletonii	P. myriotylum	P. ?oedochilum	P. splendens	P. ultimum var. sporangiferum	P. sp. J7	P. sp. J9	R. oligosporus
EUCALYPTUS SPP.																											
E. cypellocarpa	*	*	N	*	*	*	*	*	*	*	*	*	*	N	N	*	*	*	*	N	*	N	*	N	*	*	N
E. grandis	*	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*	*	*	*	*	*	N
E. maculata	*	N	N	*	*	*	*	*	*	*	*	N	*	N	N	*	*	*	*	N	*	N	*	N	*	*	N
E. marginata	*	*	*	N	N	*	*	*	*	N	N	*	*	N	N	*	*	N	*	N	N	N	N	N	N	N	N
E. pilularis	*	*	N	*	*	*	*	*	*	*	*	*	*	N	N	*	*	*	*	*	*	*	*	N	*	*	N
E. regnans	*	*	N	*	*	*	*	*	*	*	N	*	*	N	N	*	*	*	*	N	*	N	*	N	*	*	N
E. sieberi	*	*	*	*	*	*	*	*	*	*	*	*	*	N	N	*	*	*	*	*	*	*	*	N	*	*	N
E. viminalis	*	*	*	*	*	*	*	*	*	*	*	*	*	N	N	*	*	*	*	*	*	*	*	N	*	*	N
PINUS SPP.																											
P. caribaea	*	N	N	*	N	*	*	*	*	*	*	*	*	N	N	*	N	N	*	N	*	N	N	N	N	N	N
P. elliottii	*	*	*	*	*	*	*	*	*	*	*	*	*	N	*	N	N	N	*	N	*	N	*	N	N	N	N
P. pinaster	*	*	N	*	N	N	*	*	*	N	N	*	*	N	N	*	N	N	*	N	N	N	N	N	N	N	N
P. radiata	*	*	N	*	N	N	*	*	*	N	N	*	*	N	N	*	N	N	*	N	N	N	N	N	N	N	N

N = For the particular tree species there is no significant difference between the fungal treatments and control at the 5% level.

* = Significantly different at the 5% level.

TABLE 2.6 Analysis of Variance for Proportion of *Pinus* Seedlings Emerged. Experiments 1 and 2

Source of Variation	Degrees of Freedom	Mean Square	Variance (1)	Ratio (2)
Experiment 1				
Fungi	11	.31		16.72
Trees	3	4.73		254.99 ***
Fungi x Trees	33	.05	3.47 ***	
Error (Within Sub-class	288	.015		
Error (Interaction +	321	.019		
Error (Within Sub-class				
Experiment 2				
Fungi	16	2.24		72.57
Trees	3	1.14		36.76 ***
Fungi x Trees	48	.15	9.36 ***	
Errors (Within Sub-class	408	.016		
Errors (Interaction +	456	.031		
Errors (Within Sub-class				

*** Significant at the 0.01% level

(1) Variance Ratio. Interaction/Within Sub-class

(2) Variance Ratio. Main Effects/Interaction + Within Sub-class

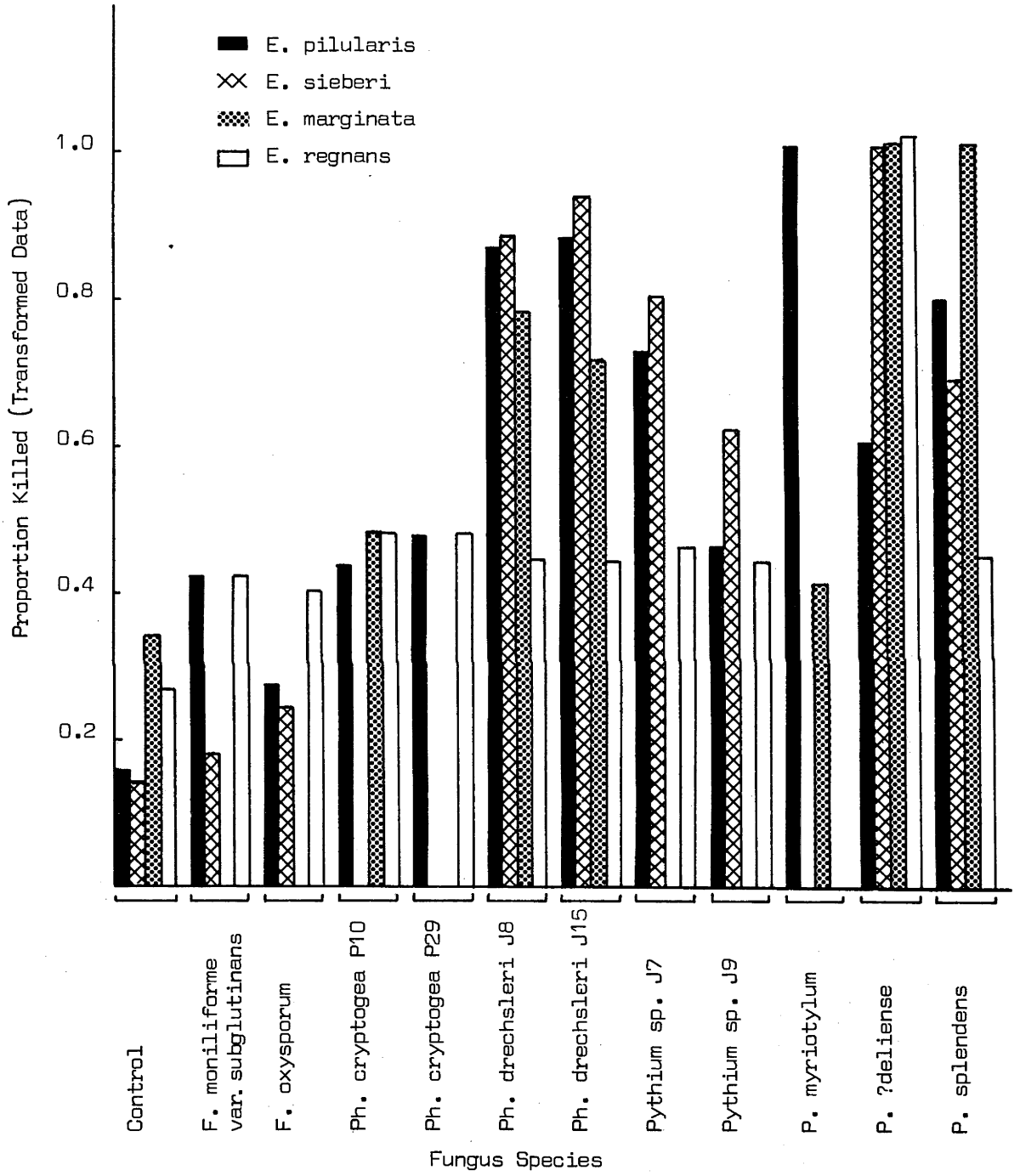

FIG. 2.7 Proportion of Eucalypt Seedlings Killed. Experiment No. 1

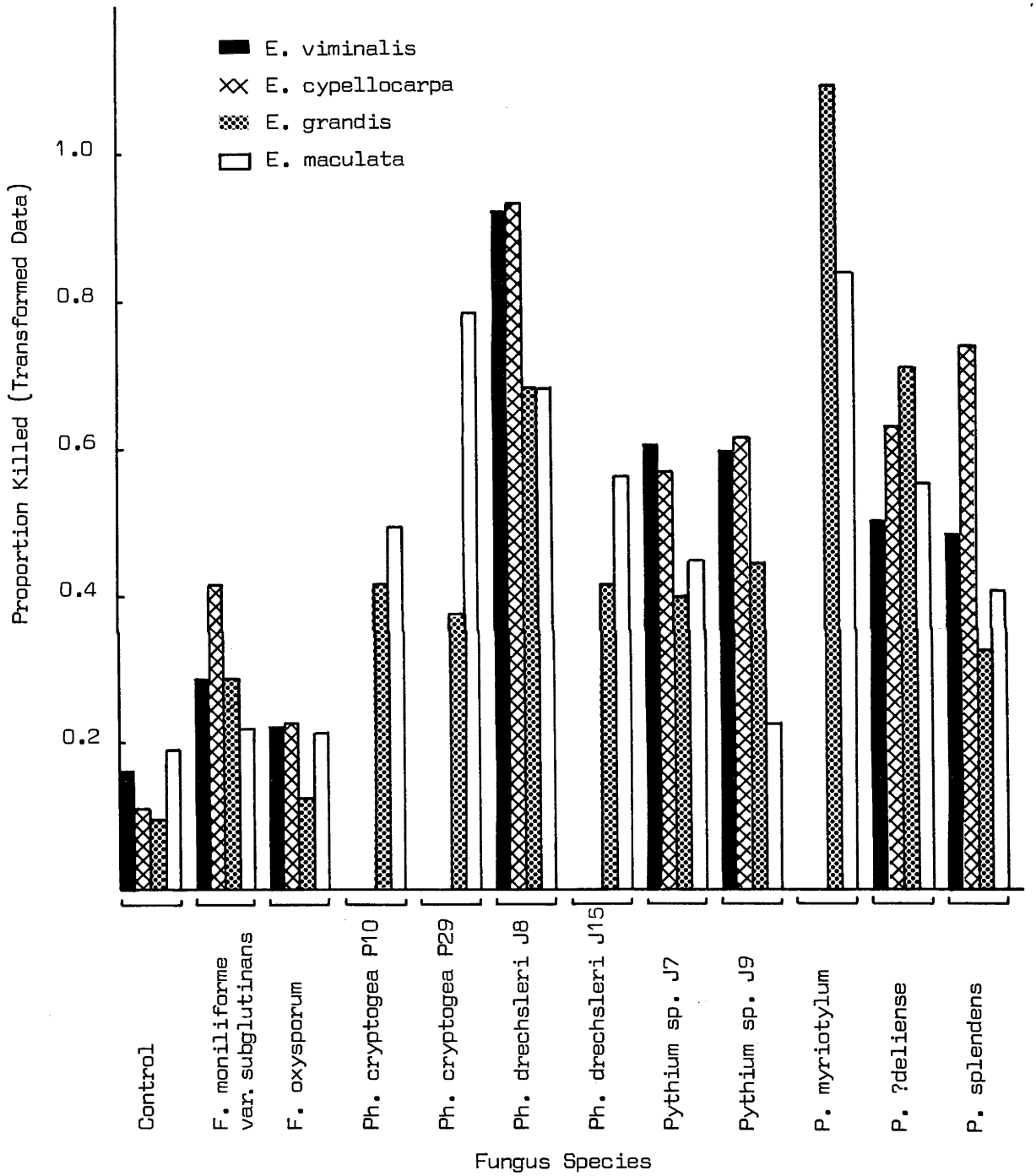

FIG. 2.8 Proportion of Eucalypt Seedlings Killed.
Experiment No. 1

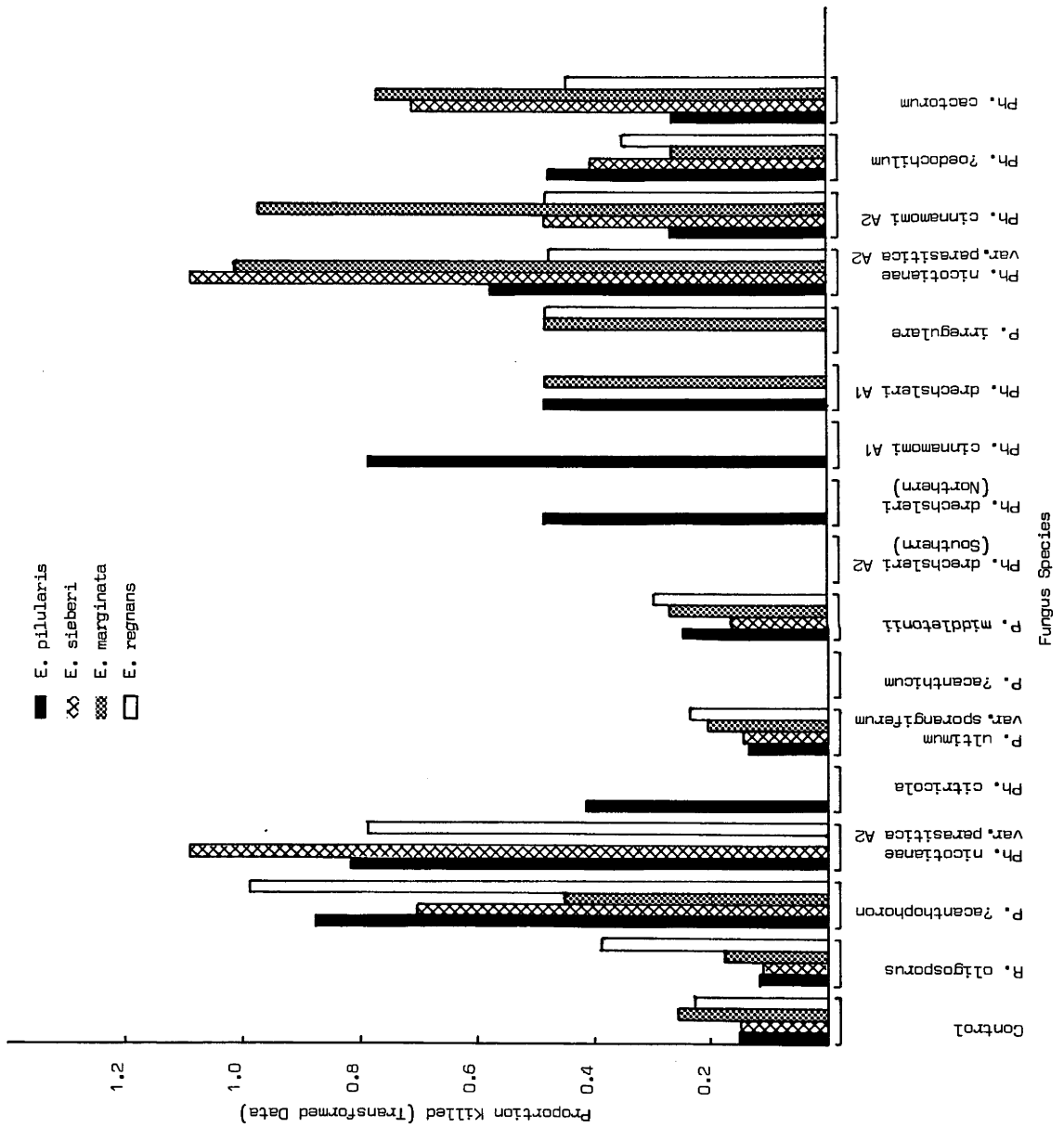

FIG. 2.9 Proportion of Eucalypt Seedlings Killed. Experiment No. 2

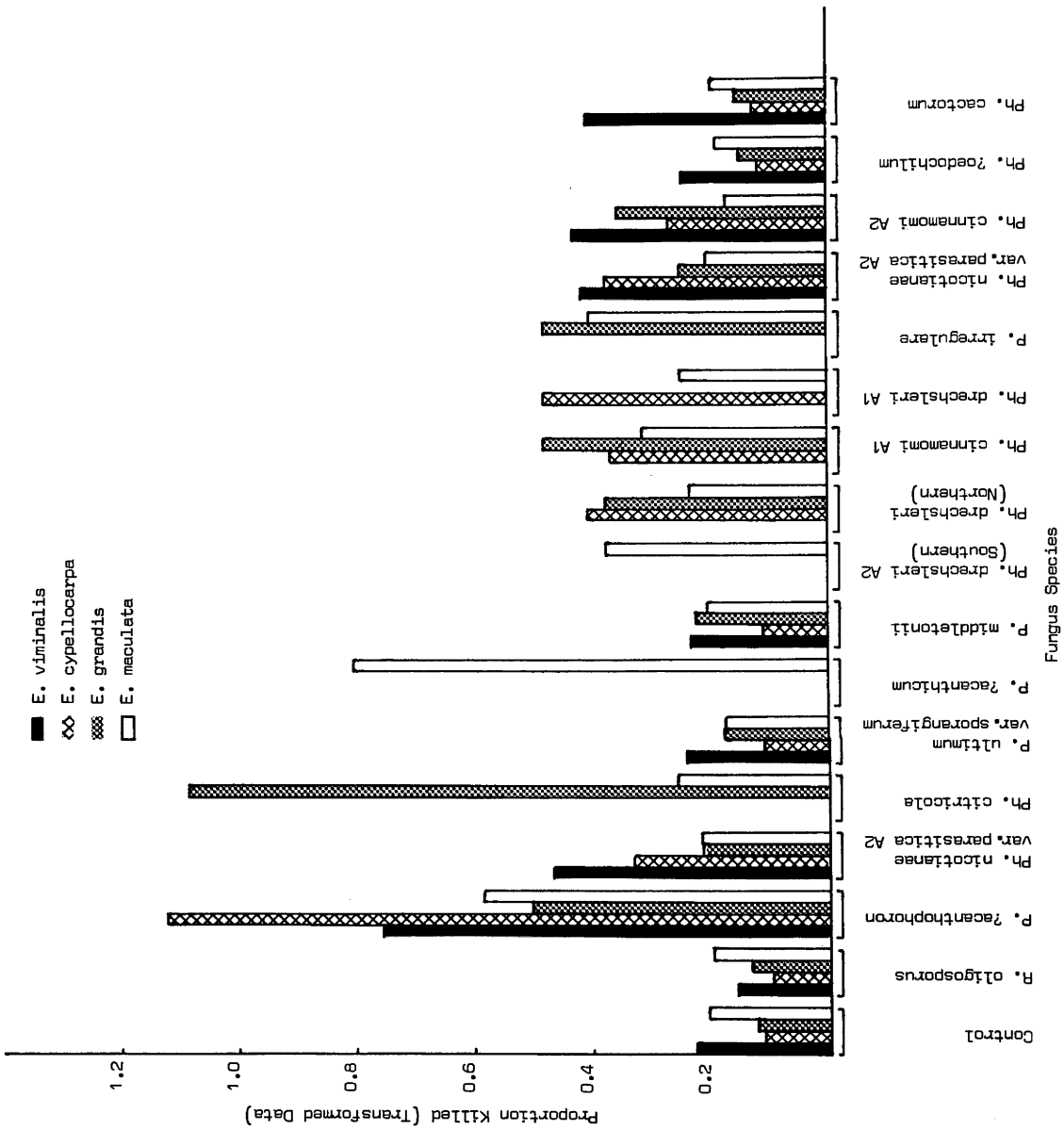

FIG. 2.10 Proportion of Eucalypt Seedlings Killed. Experiment No. 2

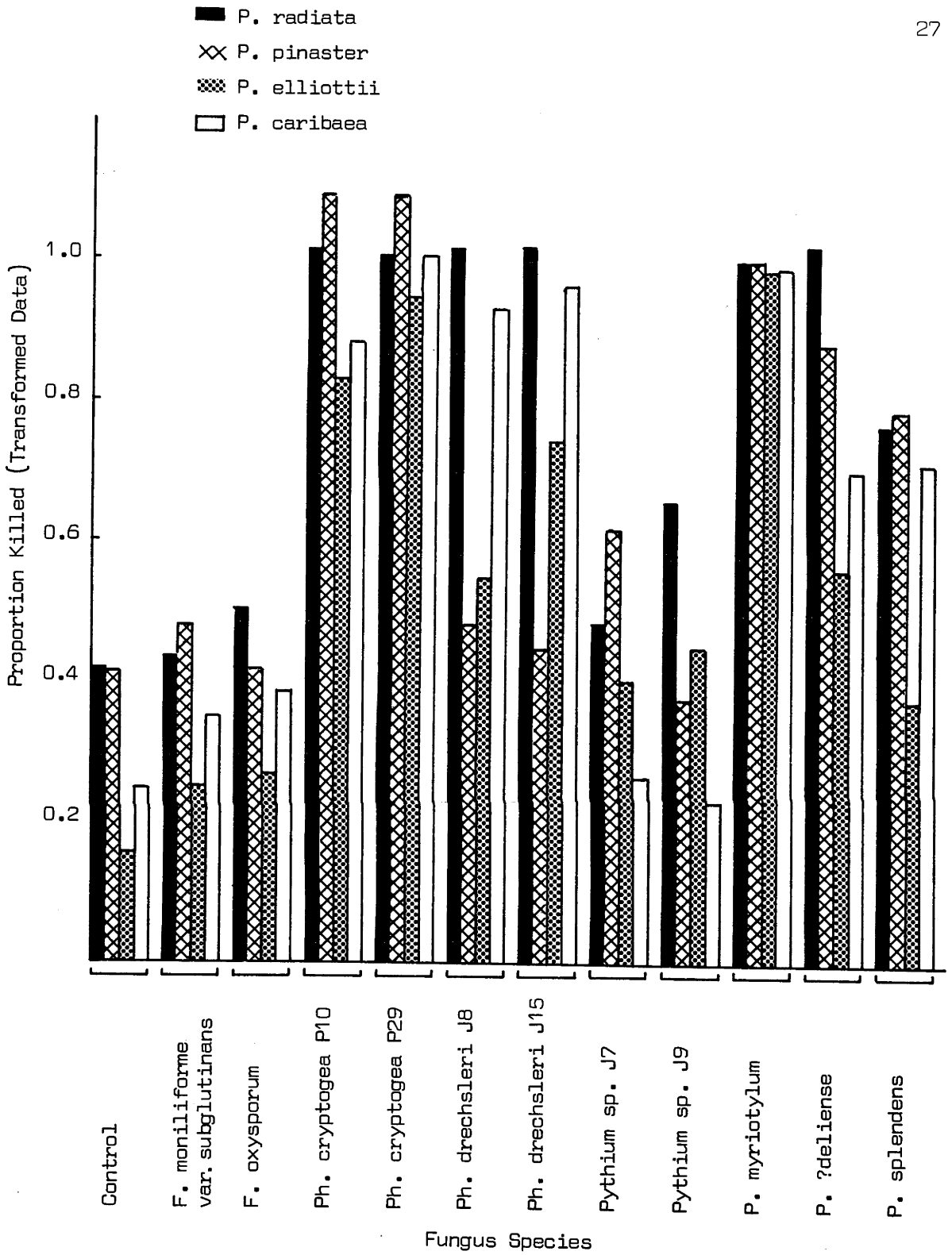

FIG. 2.11 Proportion of Pinus Seedlings Killed. Experiment No. 1

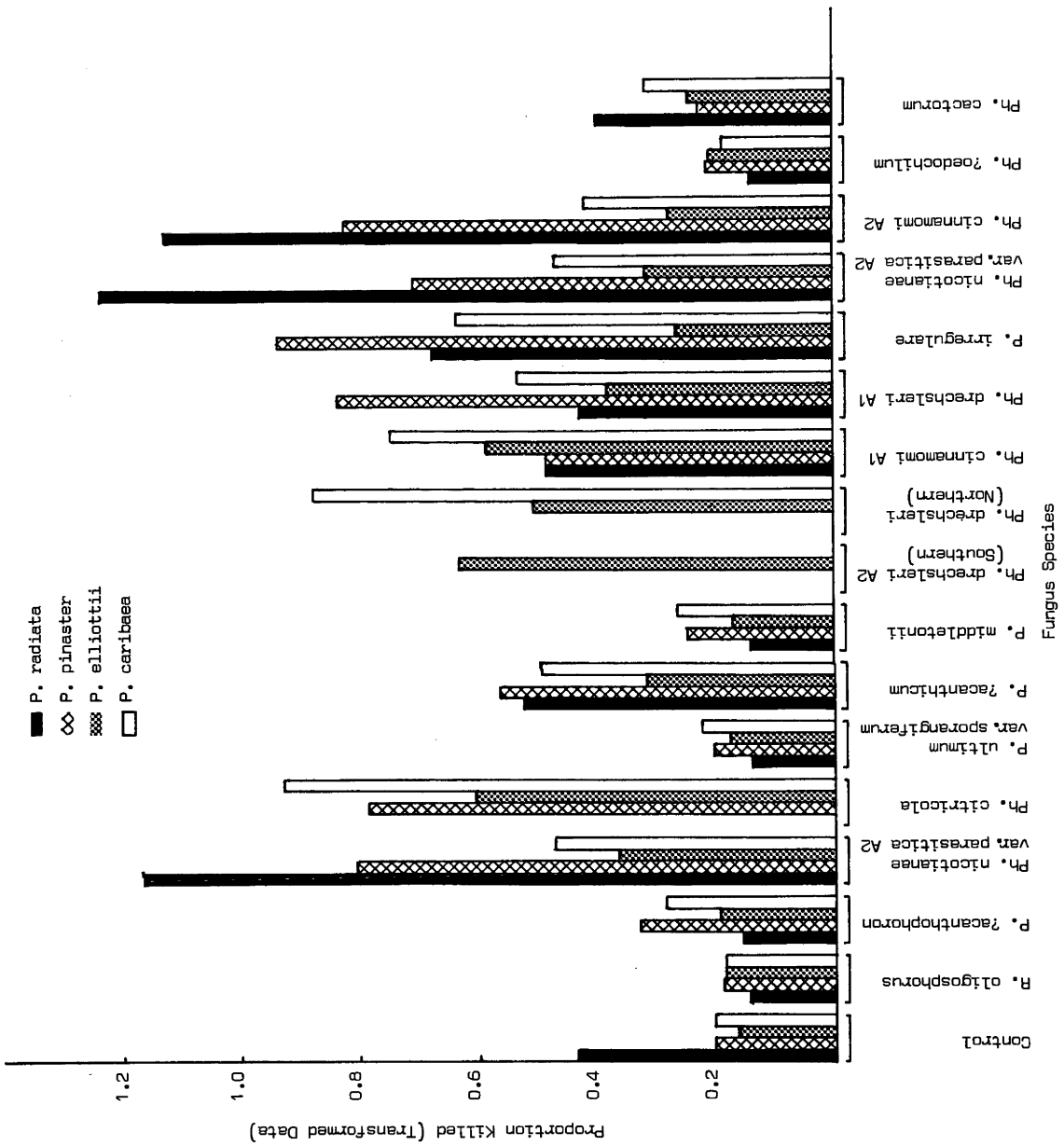

FIG. 2.12 Proportion of Pinus Seedlings Killed. Experiment No. 2

TABLE 2.7 Summary of Post-Emergence Damping-Off, Graphically Shown in Figs. 2.7 - 2.12. Comparisons Made Within a Tree Species Using LSD 5%

Fungal Species	Ph. cinnamomi A1	Ph. cinnamomi A2	Ph. cactorum	Ph. citricola	Ph. cryptogea P10	Ph. cryptogea P29	Ph. drechsleri Nth.	Ph. drechsleri A1	Ph. drechsleri K93	Ph. drechsleri J8	Ph. drechsleri J15	Ph. nicotianae var. parasitica A2 J18	Ph. nicotianae var. parasitica A2 J28	F. moniliforme var. subglutinans	F. oxysporum	P. ?acanthicum	P. ?acanthophoron	P. ?deliense	P. irregulare	P. middletonii	P. myriotylum	P. ?oedochilum	P. splendens	P. ultimum var. sporangiferum	P. sp. J7	P. sp. J9	R. oligosporus
EUCALYPTUS SPP.																											
E. cypellocarpa	*	N	N	X	X	X	*	*	X	*	X	*	*	*	N	X	*	*	X	N	X	N	*	N	*	*	N
E. grandis	*	*	N	*	*	*	*	X	X	*	*	N	N	N	N	X	*	*	*	N	*	N	N	N	*	*	N
E. maculata	N	N	*	N	*	*	N	N	N	*	*	N	N	N	N	*	*	*	N	N	*	N	*	N	*	N	N
E. marginata	X	*	*	X	*	X	X	*	X	*	*	X	*	X	X	X	*	*	N	N	*	N	*	N	X	X	N
E. pilularis	*	N	N	*	*	*	*	*	X	*	*	*	*	*	N	X	*	*	X	N	*	*	*	N	*	*	N
E. regnans	X	*	*	X	N	N	X	X	X	N	N	*	*	N	N	X	*	N	*	N	X	N	N	N	N	N	N
E. sieberi	X	*	*	X	X	X	X	X	X	*	*	*	*	N	N	X	*	*	X	N	X	*	*	N	*	*	N
E. viminalis	X	*	*	X	X	X	X	X	X	*	X	*	*	N	N	X	*	*	X	N	X	N	*	N	*	*	N
PINUS SPP.																											
P. caribaea	*	*	N	*	*	*	*	*	X	*	*	*	*	N	N	*	N	*	*	N	*	N	*	N	N	N	N
P. elliottii	*	N	N	*	*	*	*	N	*	*	*	*	N	N	N	N	N	*	N	N	*	N	N	N	*	*	N
P. pinaster	*	*	N	*	*	*	X	*	X	N	N	*	*	N	N	*	N	*	*	N	*	N	*	N	N	N	N
P. radiata	N	*	N	X	*	*	X	N	X	*	*	*	*	N	N	N	N	*	N	N	*	N	*	N	N	N	N

N = For the particular tree species there is no significant difference between the losses incurred in the treatment and the control at the 5% level.

* = Significantly different at the 5% level.

X = Zero values after germination of seed, hence post-emergence damping-off could not be assessed.

TABLE 2.8 Analysis of Variance for Proportion of Seedlings Killed. Experiments 1 and 2. (Includes Both *Eucalyptus* and *Pinus* spp.)

Source of Variation	Degrees of Freedom	Mean Square	Variance (1)	Ratio (2)
Experiment 1				
Fungi	11	2.68		49.86
Trees	11	.58		10.74 ***
Fungi x Trees	104	.12	3.01 ***	
Error (Within Sub-class	499	.04		
Error (Interaction +	603	.054		
Error (Within Sub-class				
Experiment 2				
Fungi	16	1.69		32.35
Trees	11	.74		14.15 ***
Fungi x Trees	134	.207	7.86 ***	
Error (Within Sub-class	802	.026		
Error (Interaction +	936	.052		
Error (Within Sub-class				

*** Significant at the 0.1% level

(1) Variance Ratio. Interaction/Within Sub-class

(2) Variance Ratio. Main Effect/Interaftion + Within Sub-class

and caused pre-emergence loss in most, or all the test Eucalyptus spp. (Table 2.5 and Figs. 2.1 - 2.4). Major exceptions were F. moniliforme var. subglutinans, F. oxysporum, P. ultimum var. sporangiferum, P. middletonii and R. oligosporus, all of which had little or no effect on the emergence of any Eucalyptus spp.

The Analysis of Variance used to support the Pinus spp. data, in respect of pre-emergence damping-off, is contained in Table 2.6.

Most Phytophthora isolates were pathogenic and caused pre-emergence loss in Pinus spp. (Table 2.5, Figs. 2.5 - 2.6). Fusarium and Pythium isolates were generally less pathogenic than Phytophthora isolates, although there were exceptions. P.?acanthicum, P. irregulare and P. myriotylum caused significant losses in some species.

2.3.2 Post-Emergence Damping-Off

The highly pathogenic organisms totally prevented emergence of a number of tree species, and consequently the data for post-emergence damping-off are incomplete (Table 2.7 and Figs. 2.7 - 2.12). The Analysis of Variance Table for Experiments 1 and 2 include both Eucalyptus and Pinus spp., and are contained in Table 2.8.

The emergence of a number of Eucalyptus spp. was severely inhibited by most Phytophthora isolates. For those species and isolates where data are available most Phytophthora spp. caused post-emergence loss in the majority of Eucalyptus spp. (Table 2.7 and Figs. 2.7 - 2.10). Fusarium and Pythium spp. were less pathogenic

than *Phytophthora* spp., although there were exceptions. *P.* ?*acanthophoron*, *P.* ?*deliense*, *P. myriotylum*, *P. splendens*, *Pythium* sp. (J7), and *Pythium* sp. (J9) caused some loss in most *Eucalyptus* spp.

Most *Phytophthora* spp. caused post-emergence loss in *Pinus* spp. (Table 2.7). Few *Pythium* isolates were pathogenic. However, *P.* ?*acanthicum*, *P.* ?*deliense*, *P. irregulare*, *P. myriotylum* and *P. splendens* induced post-emergence losses in some species.

All host species were susceptible to some fungi. Variation in the degree of susceptibility of tree species to pre- and post-emergence damping-off, suggests that to rank or group species on the basis of their overall resistance to disease is to oversimplify the situation. *Eucalyptus maculata*, however, was outstanding in its overall resistance.

The isolates originally used to inoculate the sand were recovered by baiting the soil water and they were recovered from the plated roots of some seedlings.

CHAPTER 3

THE INFLUENCE OF SOIL MICROFLORA ON PATHOGENICITY

3.1 INTRODUCTION

The previous chapter presented the results of pre- and post-emergence damping-off tests of a number of organisms on a range of commercial timber species, the experimental medium being a steam-air-treated sand.

In the experiment reported here unsterilised soils of differing nutrient status were used to replace the sand medium of the pathogenicity trials described in Chapter 2.

3.2 MATERIALS AND METHODS

3.2.1 The Soil

Two soils were obtained from the South Coast of New South Wales, near Eden, viz.,

1. a soil from a moist gully site supporting *Eucalyptus cypellocarpa* as the dominant overstorey species, and
2. a soil from a drier ridge site supporting *Eucalyptus sieberi* as the dominant overstorey species.

The soils were examined thoroughly for the presence of *Phytophthora* spp. using the lupin baiting technique of Pratt and Heather (1972), direct soil plating and the soil dilution plate methods of isolation. For each plating method both the medium of Eckert and Tsao (1962) and 2% water streptomycin (50 ppm) agar were used.

Lupin baiting and soil plating did not detect the presence of any *Phytophthora* spp.

Each soil was also analysed for Nitrogen and Phosphorus, using the technique of Fogg and Wilkinson (1958).

The results indicate that the levels of Nitrogen and Phosphorus are considerably higher in the soil normally supporting Eucalyptus cypellocarpa.

Soil Analysis

	Nitrogen Levels	Phosphorus (ppm.)
Soil normally supporting Eucalyptus cypellocarpa	975	70
Soil normally supporting Eucalyptus sieberi	350	10

3.2.2 The Fungal Species

The following organisms, examined previously for pathogenic ability (Chapter 2), were used for further pathogenicity testing in a nonsterile system.

Fusarium moniliforme var. subglutinans

Fusarium oxysporum

Phytophthora cryptogea (P29)

Phytophthora nicotianae var. parasitica (A2 IMI 168069)

Pythium ?acanthicum

Phythphthora drechsleri (Northern)

Phytophthora cinnamomi (A1)

Phytophthora drechsleri (A1)

Pythium irregulare

Phytophthora cinnamomi (A2)

Phytophthora drechsleri (A2 Southern K24)

3.2.3 The Tree Species

The following species were used as test hosts.

Eucalyptus cypellocarpa

Eucalyptus maculata

Eucalyptus pilularis

Eucalyptus regnans

Eucalyptus sieberi

Eucalyptus viminalis

Two species tested previously, Eucalyptus marginata and Eucalyptus grandis, were not used in this trial because of problems of seed viability, seedling size and slow seedling development.

3.2.4 Experimental Procedure

Inoculum, seed, cups and trays were prepared as before (Section 2.2).

The Experimental Procedure described (Section 2.2.6) was amended as follows.

Periodic baiting of the soil water was not carried out. However, at the commencement and termination of the experiment the soil water was baited with lupins in an attempt to recover the fungus used in the original inoculation. Also at the conclusion of the experiment root pieces from living plants in each soil, and for each test organism, were plated on both 2% water streptomycin (50 ppm) agar and the medium of Eckert and Tsao (1962).

3.2.5 Statistical Analysis

Three factors were considered in the analysis: viz. Soil, Tree species and Fungal species. The data were analysed in two parts.

3.2.5.1 Number of Seedlings Emerged : Pre-Emergence Damping-Off

Data were transformed using a square root transformation, the analysis being an orthogonal 3 factor factorial with internal replication.

3.2.5.2 Proportion of Seedlings Killed : Post-Emergence Damping-Off

Data were transformed using the arcsine transformation (Section 2.2.6), the analysis being a 3 factor non-orthogonal factorial.

3.3 RESULTS

The basic data are described in Appendix 3. The transformed data are best considered in two parts.

3.3.1 Pre-Emergence Damping-Off

The data show that the number of seedlings emerged varied significantly with change in pathogen, soil and tree species (Table 3.1). Two of the three first order interactions are significant viz., Soil x Fungi and Tree x Fungi. These interactions are plotted (Figs. 3.1, 3.2 and 3.3).

The ability of a fungus to cause pre-emergence damping-off loss is dependent on the soil. *P.?acanthicum*, *P. irregulare*,

TABLE 3.1 Analysis of Variance for Number of Seedlings Emerged (Pre-Emergence Damping-Off)

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio (1)	Ratio (2)
Fungi	11			
Trees	5			
Soil	1			
Soil x Fungi	11	3.424	7.98***	88.83 ***
Soil x Trees	5	.719	1.68 NS	
Trees x Fungi	55	13.150	30.65***	
Residual	919	.429		

*** Significant at the 0.1% level.

NS Not Significant

(1) Variance Ratio. 1st Order Interaction/Residual

(2) Variance Ratio. Main Effects + 1st Order Interactions compared with the Residual

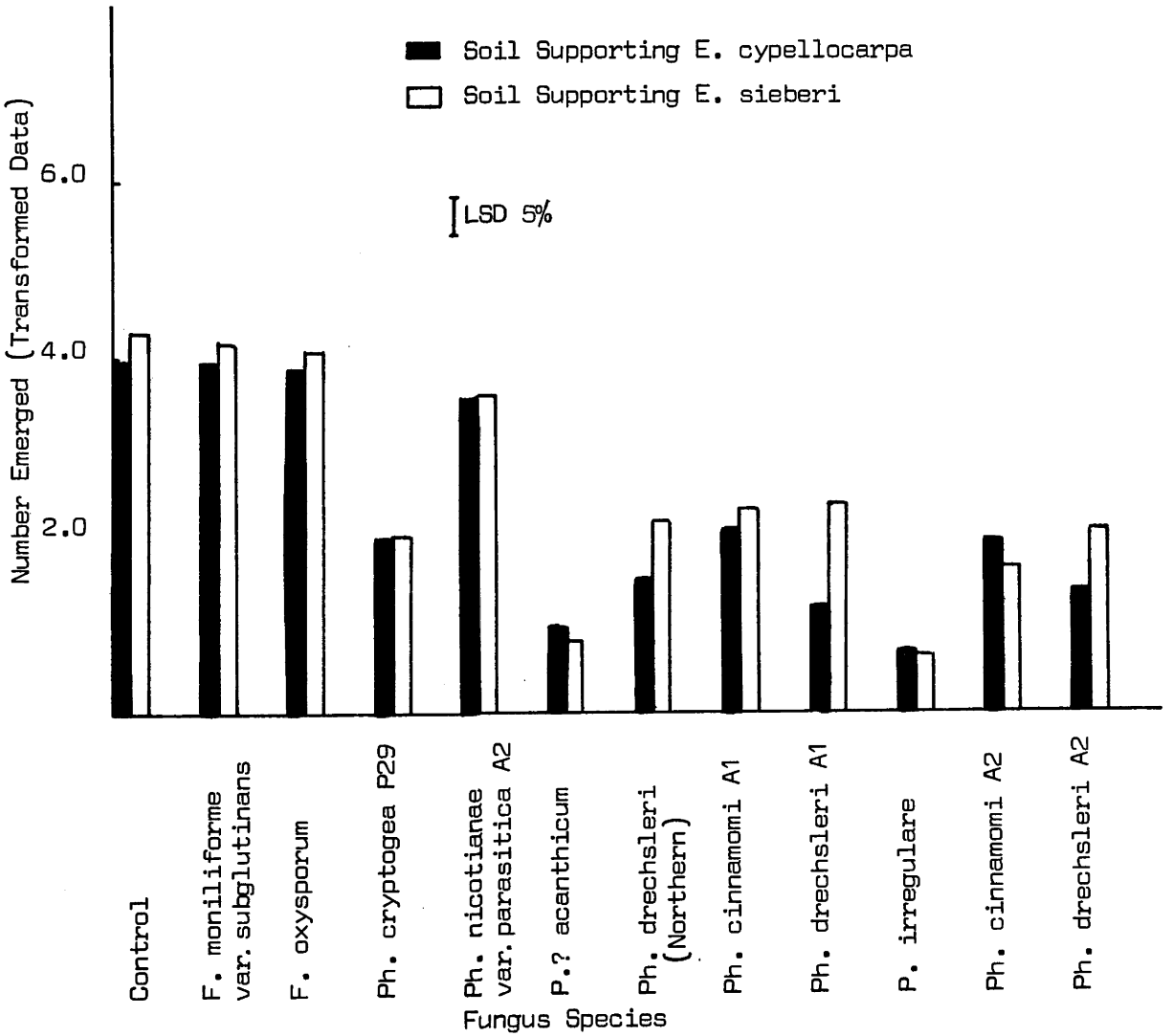

FIG. 3.1 Number of Seedlings Emerged. Soil x Fungi Interaction

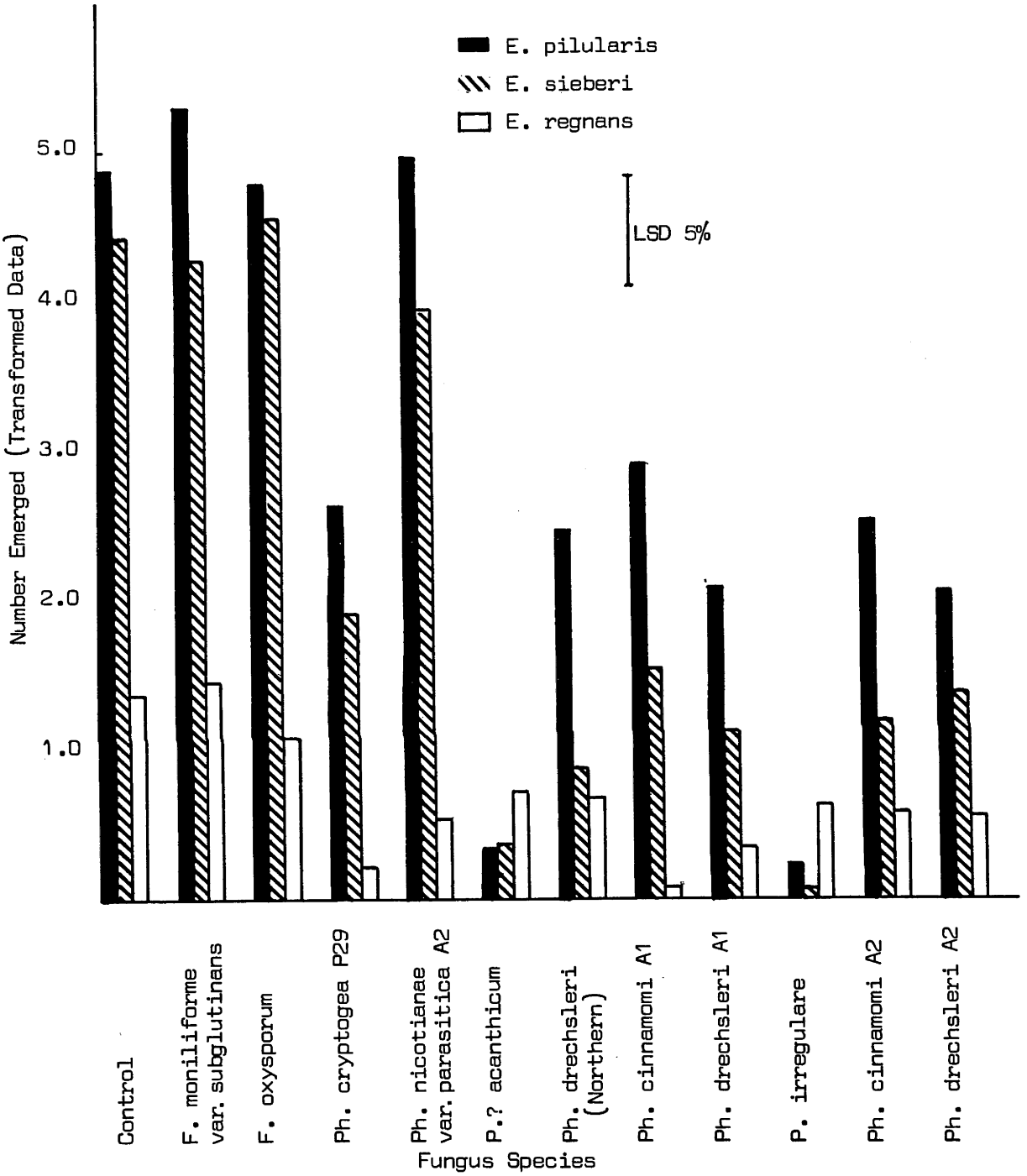

FIG. 3.2 Number of Seedlings Emerged. Tree x Fungi Interaction

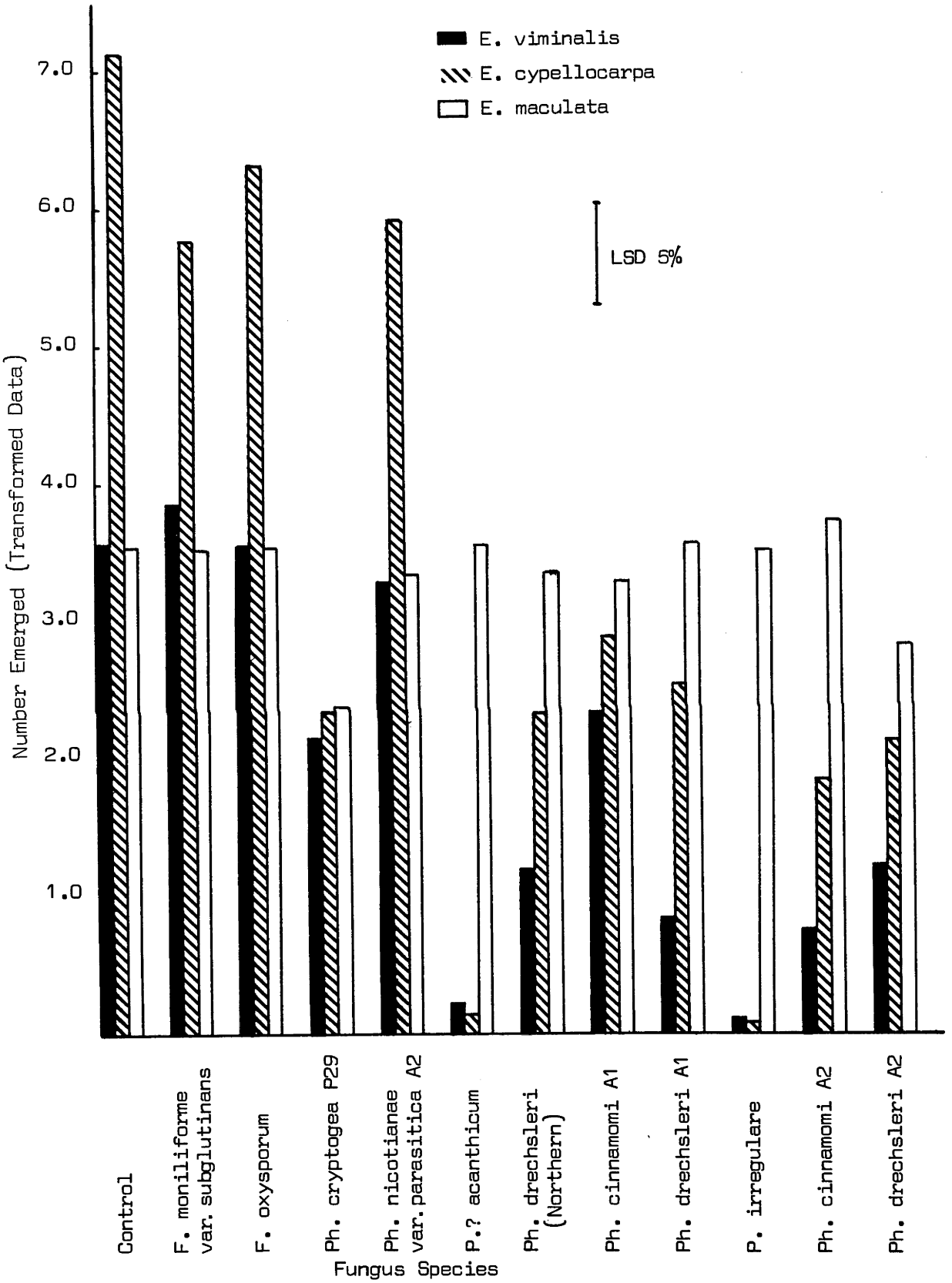

FIG. 3.3 Number of Seedlings Emerged. Tree x Fungi Interaction

TABLE 3.2 LSD 5% Used to Compare the Effects of the Introduced Fungus with the Control for Individual Tree Species. Summary of Figs. 3.2 and 3.3 (Pre-Emergence Damping-Off)

Fungal Species	*F. moniforme* var. *subglutinans*	*F. oxysporum*	*Ph. cryptogea* (P10)	*Ph. nicotianae* var. *parasitica* (A2)	*P. ?acanthicum*	*Ph. drechsleri* (Nth.)	*Ph. cinnamomi* (A1)	*Ph. drechsleri* (A1)	*P. irregulare*	*Ph. cinnamomi* (A2)	*Ph. drechsleri* (A2)
Tree Species											
E. pilularis	N	N	*	N	*	*	*	*	*	*	*
E. sieberi	N	N	*	N	*	*	*	*	*	*	*
E. regnans	N	N	*	*	*	*	*	*	N	*	*
E. viminalis	N	N	*	N	*	*	*	*	*	*	*
E. cypellocarpa	*	N	*	*	*	*	*	*	*	*	*
E. maculata	N	N	*	N	N	N	N	N	N	N	*

N = Treatment with the introduced fungus not significantly different from the control at the 5% level.

* = Treatment significantly different from the control at the 5% level.

TABLE 3.3 Analysis of Variance for Proportion of Seedlings Killed (Post-Emergence Damping-Off)

Source of Variation	Degrees of Freedom	Mean Square	Variance (1)	Ratio (2)
Fungi	11			
Trees	5			
Soil	1			
Soil x Fungi	11	.864	1.52 NS	14.80 ***
Soil x Trees	5	.269	6.44***	
Trees X Fungi	55	.250	5.99***	
Residual	702	.042		

*** Significant at the 0.1% level.

NS Not Significant

(1) Variance Ratio. 1st Order Interaction/Residual

(2) Variance Ratio. Main Effects + 1st Order Interactions compared with the Residual

Ph.cinnamomi (A1 and A2 isolates), Ph. cryptogea and Ph. drechsleri (Northern, A1 and A2 isolates) caused considerable loss in both soils and for most tree species (Fig. 3.1). The Fusarium spp. and Ph. nicotianae var. parasitica exhibited limited pathogenicity.

The Least Significant Difference (LSD 5%) was used to test for significant differences between soils for individual fungi (Fig. 3.1). It confirmed observations that all three isolates of Ph. drechsleri demonstrated an increased pathogenicity in the soil normally supporting Eucalyptus cypellocarpa.

Significant differences between fungal treatments for a particular tree species are considered. Table 3.2 has been constructed using the LSD 5% shown in Figs. 3.2 and 3.3. Loss attributable to pre-emergence damping-off depended upon tree species. Eucalyptus maculata offered some resistance to infection by most organisms. Ph. cryptogea and Ph. drechsleri (A2 Southern) were the only organisms to cause significant pre-emergence damping-off in this species.

3.3.2 Post-Emergence Damping-Off

The data show that the 'Proportion of Seedlings Killed' varied significantly with change in pathogen, soil and tree species (Table 3.3). Again two of the three first order interactions are significant viz., Soil x Tree and Tree x Fungi (Figs. 3.4, 3.5 and 3.6).

Post-emergence damping-off losses were less for Eucalyptus maculata (Fig. 3.4) than for any of the other Eucalyptus spp. used.

Further consideration of the Soil x Tree interaction (Fig. 3.4) is not warranted. The non-orthogonal nature of the analysis make other comparisons of limited use.

Loss caused by post-emergence damping-off varied with pathogen and tree species (Fungi x Tree Interaction, Figs. 3.5 and 3.6). Table 3.4 has been constructed using the LSD 5% shown on these graphs. Eucalyptus maculata did not show any significant loss from post-emergence damping-off by any organism.

With few exceptions Fusarium spp., Ph. nicotianae var. parasitica and P. irregulare did not cause post-emergence damping-off in most Eucalyptus spp.

The organisms used to inoculate the soils were recovered from the soil water. Recovery of the organism used in the original inoculation from platings of root pieces was less successful than under the sterile conditions described in Chapter 2.

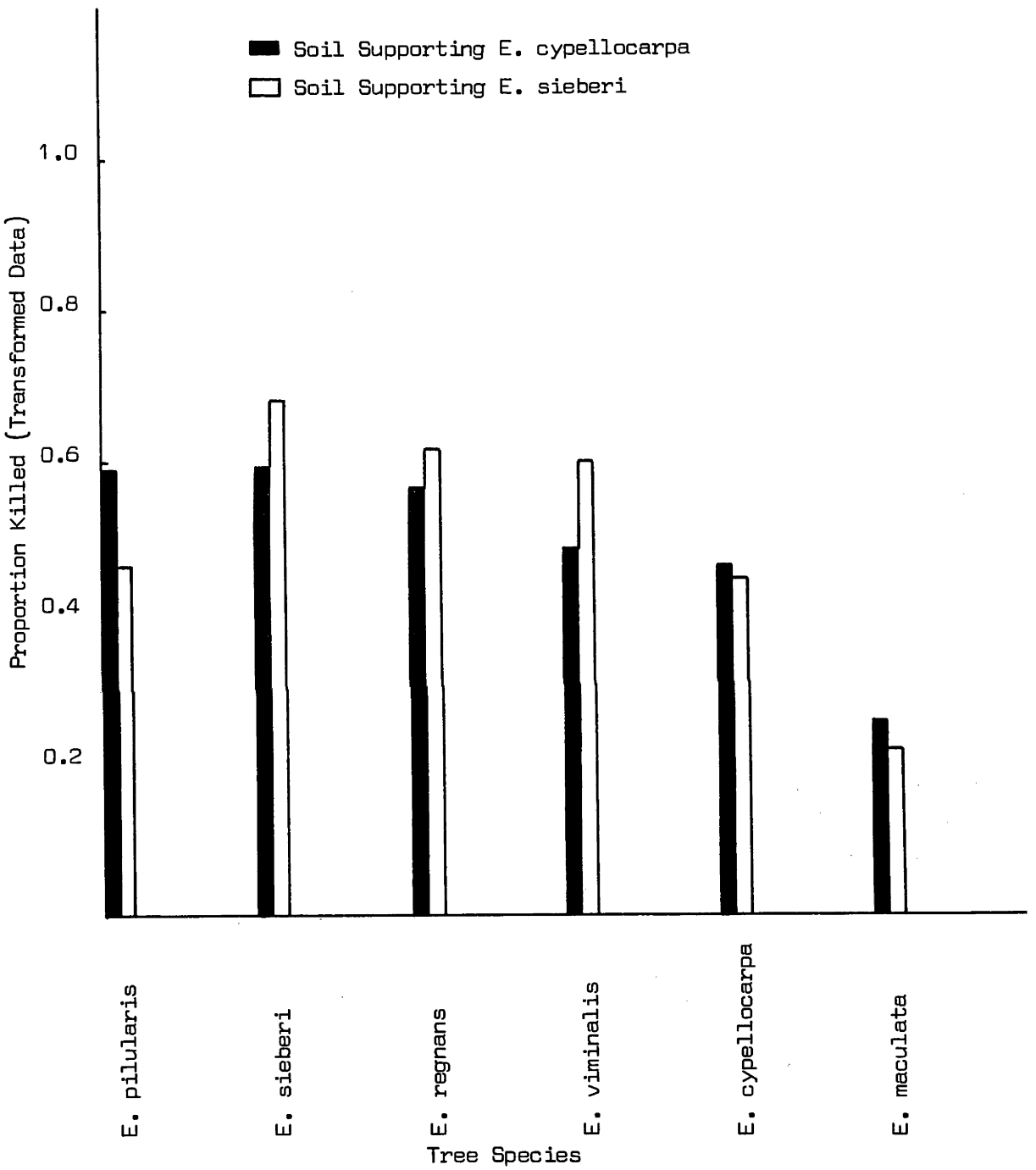

FIG. 3.4 Proportion of Seedlings Killed. Soil x Tree Interaction

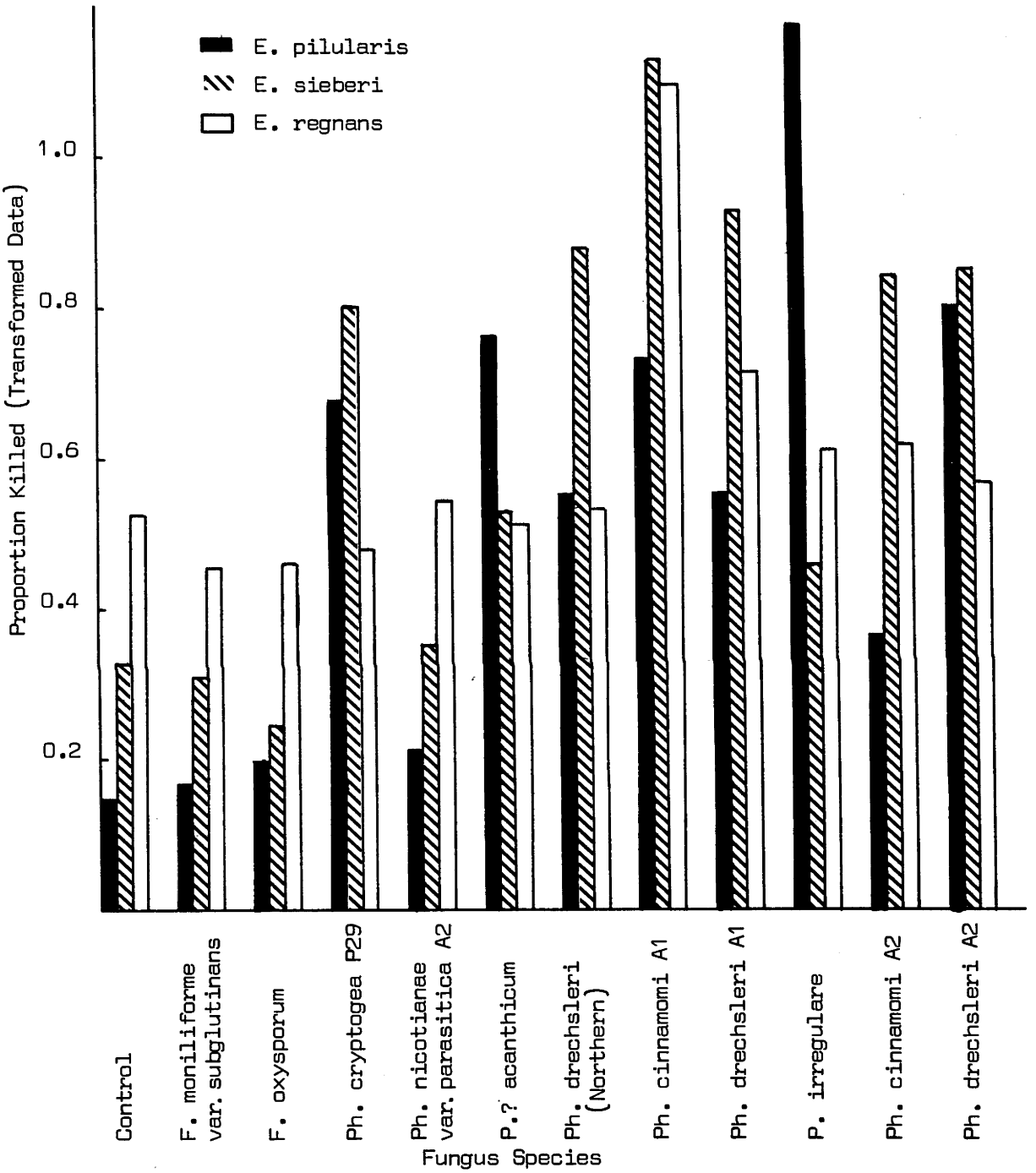

FIG. 3.5 Proportion of Seedlings Killed. Tree x Fungi Interaction

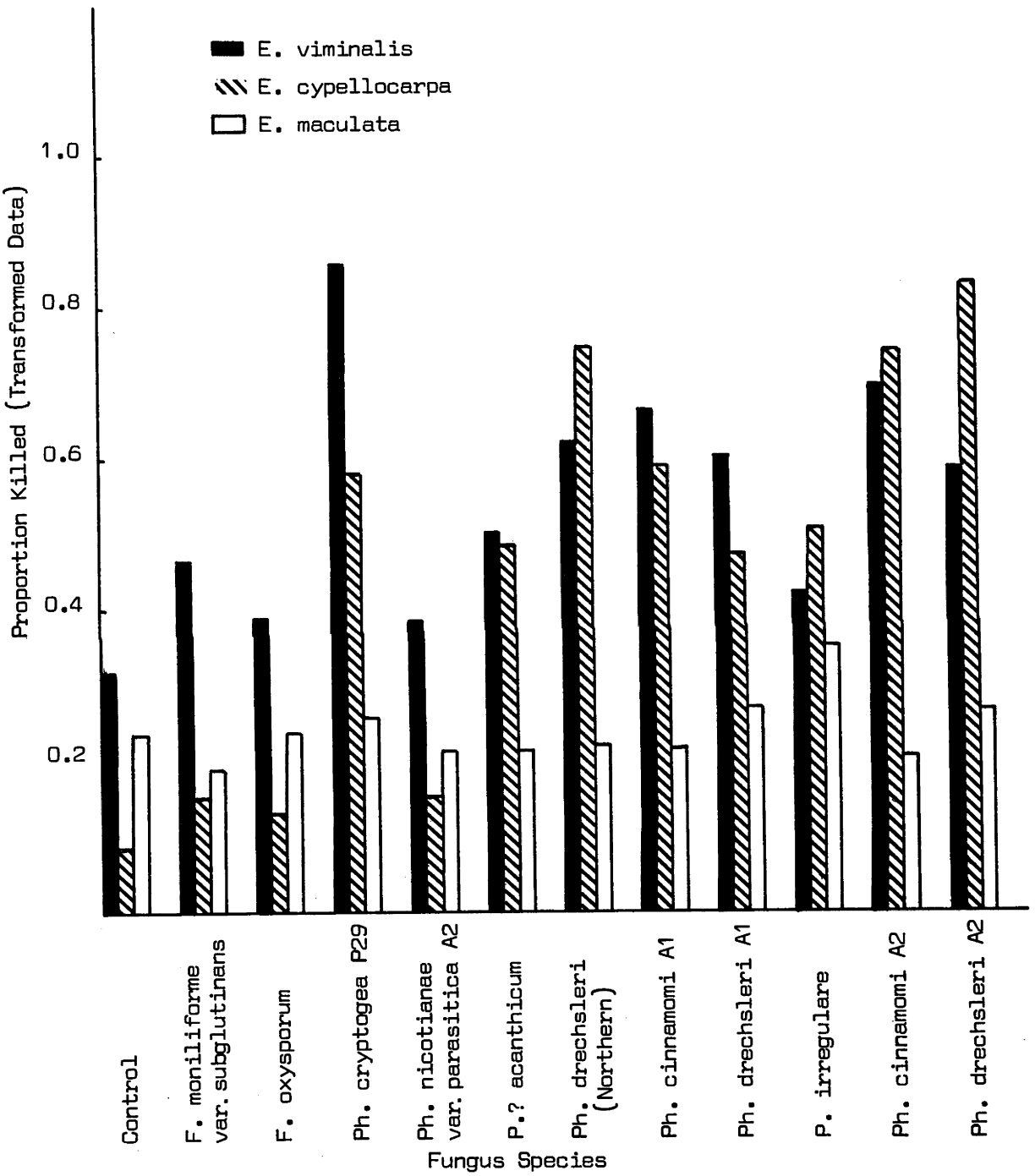

FIG. 3.6 Proportion of Seedlings Killed. Tree x Fungi Interaction

TABLE 3.4 LSD 5% Used to Compare the Effect of the Introduced Fungus with the Control for Individual Tree Species. Summary of Figs. 3.5 and 3.6 (Post-Emergence Damping-Off)

Fungal Species	*F. moniliforme* var. *subglutinans*	*F. oxysporum*	*Ph. cryptogea* (P10)	*Ph. nicotianae* var. *parasitica* (A2)	*P.? acanthicum*	*Ph. drechsleri* (Nth.)	*Ph. cinnamomi* (A1)	*Ph. drechsleri* (A1)	*P. irregulare*	*Ph. cinnamomi* (A2)	*Ph. drechsleri* (A2)
Tree Species											
E. pilularis	N	N	*	N	*	*	*	*	*	N	*
E. sieberi	N	N	*	N	*	*	*	*	N	*	*
E. regnans	N	N	N	N	N	N	*	*	N	N	N
E. viminalis	N	N	*	N	*	*	*	*	N	*	*
E. cypellocarpa	N	N	*	N	*	*	*	*	*	*	*
E. maculata	N	N	N	N	N	N	N	N	N	N	N

N = Treatment with the introduced fungus not significantly different from the control at the 5% level.

* = Treatment significantly different from the control at the 5% level.

CHAPTER 4

THE INFLUENCE OF SOIL-BORNE PATHOGENS ON PATHOGENICITY

4.1 INTRODUCTION

The data presented in Chapter 3 indicated that under the experimental conditions then used, the natural biotic, chemical and physical components of the soils did not detract from or enhance the pathogenicity of any fungus on any tree species, with the exception of Ph. drechsleri and Ph. nicotianae var. parasitica.

Broadbent and Baker (1974) have indicated that some soils are suppressive and others conducive to root rot by Ph. cinnamomi. Hence, the existence of Phytophthora or Pythium spp. within a field soil might be associated with differences in receptivity of that soil to an introduced species, either identical or related to that originally occurring.

This study examines the pathogenicity of a number of fungi introduced into three soils that were known to be different in respect to the Phytophthora spp. naturally contained within them.

The effect of aqueous extracts from different soils on sporangial formation of the Phytophthora spp. was also studied.

4.2 MATERIALS AND METHODS

4.2.1 The Soil

Three soils were used in this study:

1. a rain forest soil from southern New South Wales, near Wonboyn.

2. a soil known to contain Ph. cinnamomi, from Backhouse Creek, near Batemans Bay, New South Wales.

3. a soil known to contain Ph. drechsleri, from Tallaganda State Forest, near Captains Flat, New South Wales.

All three soils were baited using New Zealand blue lupins, according to the method of Pratt and Heather (1972). Also the dilution plate and soil plate methods of isolation (using both the medium of Eckert and Tsao (1962) and 2% water streptomycin (50 ppm) agar) were used to ascertain the presence of Ph. cinnamomi and Ph. drechsleri.

Ph. cinnamomi and Ph. drechsleri were isolated from the Backhouse Creek and Tallaganda soils, respectively. They were the only two Phytophthora spp. isolated, their isolation being achieved only by the use of the lupin baiting technique.

4.2.2 The Fungal Species

The following fungi were selected for further study.

Phytophthora cinnamomi A1

Phytophthora cinnamomi A2

*Phytophthora drechsleri (Northern)

Phytophthora drechsleri A1

*Phytophthora drechsleri A2 (Southern)

Phytophthora nicotianae var. parasitica A1

Phytophthora nicotianae var. parasitica A2

The source of each isolate is detailed in Table 2.1.

*The Ph. drechsleri isolates were typed according to the categories of Shepherd and Pratt (1973).

Typing of all A1 and A2 cultures was undertaken by pairing with reference cultures of the opposite strain of each species. Plugs of each culture, grown on V8 agar, were placed 2 cm. apart on a V8 agar plate and the paired cultures incubated at 25°C. When these cultures had grown together, the area in which the union had initially occurred was examined for the presence of oospores.

4.2.3 The Tree Species

The species selected were -

Eucalyptus cypellocarpa

Eucalyptus grandis

Eucalyptus maculata

Eucalyptus pilularis

Eucalyptus sieberi

Eucalyptus viminalis

Seed lot and source of each species are detailed in Table 2.3.

4.2.4 Experimental Procedure

4.2.4.1 Experiment 1. The influence of indigenous soil-borne pathogens on the pathogenicity of introduced species

Preparation of the inoculum, seed and trays were as described in Section 2.2. The experimental procedure was that described in Sections 2.2.6 and 3.2.4 with the following modifications.

Each treatment (introduction of a fungus) was duplicated, and within each treatment the number of cups of each tree species reduced from 7 to 3. Allocation of treatments to trays was completely randomised.

At the conclusion of the experiment, the soils were baited using the method of Pratt and Heather (1972). Platings of roots of seedlings, from both dead and living plants, were made on both the medium of Eckert and Tsao (1962) and 2% water streptomycin (50 ppm.) agar.

Isolates recovered were identified. The mating strains were determined using the technique described in Section 4.2.1.

4.2.4.2 Experiment 2. The effect of soil extract on sporangial formation

Each species described in Section 4.2.1 was grown on V8 agar plates, for 3 days at 25°C. From the extremities of each fungal culture 30 plugs were cut and 5 plugs were placed in each of 6 petri dishes.

Soil extracts were prepared by placing 200 gm. of soil in a flask with 1.5 l. of glass distilled water, thoroughly mixing and then allowing it to incubate at 25°C. for 24 h. The supernatant in each flask was filtered under suction through Whatman No. 1 filter paper.

To each of the prepared petri dishes was added 20 ml. of the filtered soil extract. For each fungus in each soil extract two plates were allocated. The plates were incubated at 25°C.

The mean number of sporangia formed by each isolate, in each of the soil extracts, was assessed at 12 h. and 24 h. Assessment of sporangial production was based on the categories described by Shirley (1970).

4.2.5 Statistical Analysis

4.2.5.1 Experiment 1.

A three factor orthogonal analysis was carried out on both variables,

1. Number of Seedlings Emerged (Pre-Emergence Damping-Off) being transformed using a square root transformation; and
2. Proportion of Seedlings Killed (Post-Emergence Damping-Off) being transformed using the arcsine transformation mentioned in Section 2.2.6.

In this experiment there was both external and internal replication and the design was completely randomised. Each variable was constructed as the mean of the three transformed internal replicates.

4.2.5.2 Experiment 2.

A two way Analysis of Variance with two replicates per cell was performed on the data, with the error mean square used to test the interaction.

4.3 RESULTS

4.3.1 Experiment 1.

4.3.1.1 Pre-Emergence Damping-Off

The data show that the number of seedlings emerged varied significantly with change in pathogen, soil and tree species. All three first order interactions are significant for 'Number of Seedlings Emerged' (Table 4.1). The original data may be found in Appendix 3 and the transformed data for the first order interactions are presented graphically in Figs. 4.1 - 4.4.

TABLE 4.1 Analysis of Variance for Number of Seedlings Emerged (Pre-Emergence Damping-Off)

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Soil	2	5.93	17.41 ***
Fungi	7	30.63	89.94 ***
Tree	5	41.64	122.27 ***
Soil x Fungi	14	1.22	3.58 ***
Soil x Tree	10	1.43	4.21 ***
Fungi x Tree	35	1.46	4.29 ***
Soil x Fungi x Tree	70	0.34	1.00 NS
Reference	144	0.34	

*** Significant at the 0.1% level.

NS Not Significant.

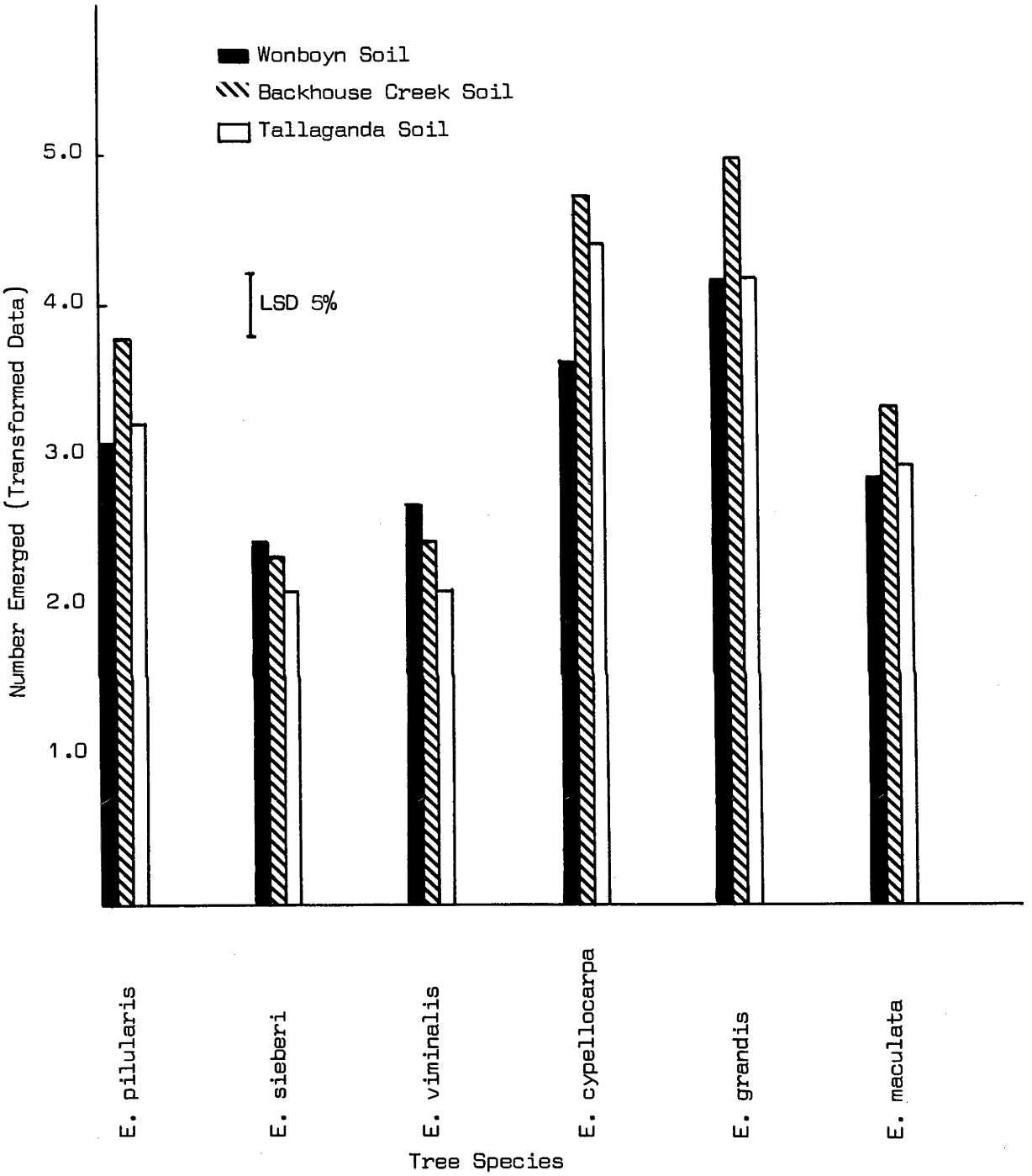

FIG. 4.1 Number of Seedlings Emerged. Soil x Tree Interaction

TABLE 4.2 Effect of Soil Type on Seedling Emergence. Using the LSD 5% Shown for Soil x Tree Interaction a Comparison Between Soils was Made

Tree Species	Soil		
	Wonboyn	Backhouse Ck.	Tallaganda
E. pilularis	-	*	N.S.
E. sieberi	-	N.S.	N.S.
E. viminalis	-	N.S.	*
E. cypellocarpa	-	*	*
E. grandis	-	*	N.S.
E. maculata	-	N.S.	N.S.

N.S. Not significantly different from the Wonboyn soil at the 5% level.

* Significantly different from the Wonboyn soil at the 5% level.

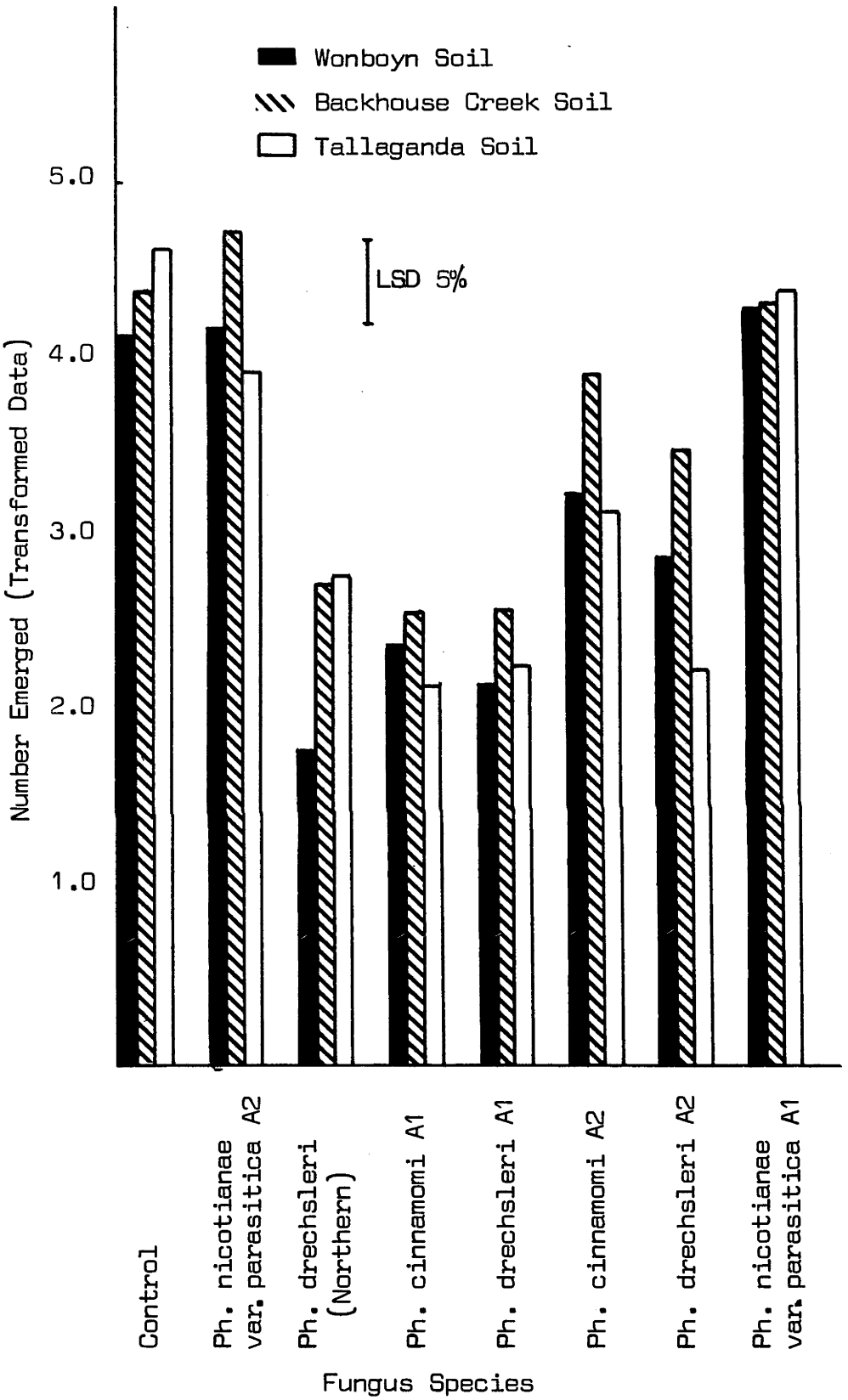

FIG. 4.2 Number of Seedlings Emerged. Soil x Fungi Interaction

TABLE 4.3 LSD 5% was Used to Compare, Within a Soil, the Effect of Introduced Fungi on the Number of Seedlings Emerged (Pre-Emergence Damping-Off). This Table is a Summary of the Soil x Fungi Interaction Fig. 4.2.

Fungal Species	Soil		
	Wonboyn	Backhouse Ck.	Tallaganda
Ph. nicotianae var. parasitica A2	N	N	*
Ph. drechsleri (Northern)	*	*	*
Ph. cinnamomi A1	*	*	*
Ph. drechsleri A1	*	*	*
Ph. cinnamomi A2	*	N	*
Ph. drechsleri A2	*	*	*
Ph. nicotianae var. parasitica A1	N	N	N

N = Not significantly different from the control at the 5% level.

* = Significantly different at the 5% level.

The disparity in numbers of seedlings emerged between each tree species in the control series and also differences in viability suggest that it is not realistic to compare directly tree species. Comparisons are therefore restricted to within tree species.

The data for the effect of soil on seedling emergence is summarized in Table 4.2. The emergence of seedlings in each of the soils is compared to that achieved in the Wonboyn soil, for each tree species. The occurrence of Ph. cinnamomi and Ph. drechsleri in the Backhouse Creek and Tallaganda soils was not associated with change in the action of introduced fungi on seedling emergence to a degree where some obvious pattern is discernable. Reduction in emergence cannot be related to the relative susceptibilities or tolerances of individual tree species.

The variation attributable to the Soil x Fungi interaction (Fig. 4.2, Table 4.3), covers the complete range of tree species used. Comparison between fungi is therefore possible. The addition of Ph. drechsleri (Northern, A1 and A2 isolates) and Ph. cinnamomi (A1 isolate) significantly reduced the emergence of seedlings in all soils. Ph. cinnamomi (A2 isolate) significantly reduced seedling emergence in the Wonboyn and Tallaganda soils.

The A1 and A2 isolates of Ph. nicotianae var. parasitica did not significantly reduce the emergence of seedlings of any Eucalyptus spp. in any of the soils, with the exception of the A2 isolate in the Tallaganda soil. In the exceptional case, the significant reduction in seedling emergence should probably be attributed to an unexplained greater activity of the indigenous

Ph. drechsleri in that soil, as that fungus was the only Phytophthora spp. isolated from diseased material (Table 4.4).

The conclusion that Ph. nicotianae var. parasitica is not pathogenic under the experimental conditions is supported by the fact that this species was only once isolated from diseased plants in the relevant series (Table 4.4). All other plants killed in those series yielded the indigenous pathogen.

Comparisons between fungi for a particular tree species can be made for the Fungi x Tree interaction from the LSD's 5% given in Figs. 4.3 and 4.4. These are summarized in Table 4.5.

Isolates of Ph. drechsleri (Northern, A1 and A2) and Ph. cinnamomi (A1 and A2) significantly reduced the emergence of seedlings of most species and could be isolated from some dying seedlings (Table 4.4). The exceptions are detailed in Table 4.5.

4.3.1.2 Post-Emergence Damping-Off

The data showed that the 'Proportion of Seedlings Killed' varied significantly with change in pathogen, soil and tree species. Two of the three first order interactions viz., Soil x Tree and Soil x Fungi, are significant (Table 4.6). The two latter are plotted in Figs. 4.5 and 4.6 respectively.

Post-emergence deaths were more numerous for most species in the Wonboyn soil (Fig. 4.5). This can be attributed to either the presence of some stimulatory factor in the Wonboyn soil which enhanced the activity of the introduced pathogen or alternatively there may have been present in the Backhouse Creek and Tallaganda soils factors which inhibited the action of the introduced pathogen, resulting in a reduction of post-emergence deaths.

TABLE 4.4 Organisms Obtained from Direct Plating of Plant Roots at the Conclusion of Pathogenicity Trials

	Soil	Tree Species					
		E. pilularis	E. sieberi	E. viminalis	E. cypellocarpa	E. grandis	E. maculata
Fungal Species							
Ph. nicotianae var. parasitica A2	a			+			
	b	(+)	(+)	(+)			
	c		(+)		(+)		
Ph. drechsleri (Northern)	a						
	b	+			+		
	c	+			+		+
Ph. cinnamomi A1	a						
	b	+			+		
	c				+		
Ph. drechsleri A1	a						
	b					(+)	
	c						
Ph. cinnamomi A2	a						
	b	(+)					
	c						
Ph. drechsleri A2 (Southern)	a	+				+	
	b	+		+	+	+	+
	c				+	+	+
Ph. nicotianae var. parasitica A1	a						
	b		(+)				
	c	(+)					
Control	a						
	b	(+)	(+)	(+)			
	c	(+)	(+)	(+)	(+)		

(+) = Organism isolated from the natural microbial population of the soil.

\+ = Inoculated pathogen re-isolated from plant roots.

a = Wonboyn Rain Forest Soil

b = Soil containing Ph. cinnamomi as a component of the natural soil population

c = Soil containing Ph. drechsleri as a component of the natural soil population

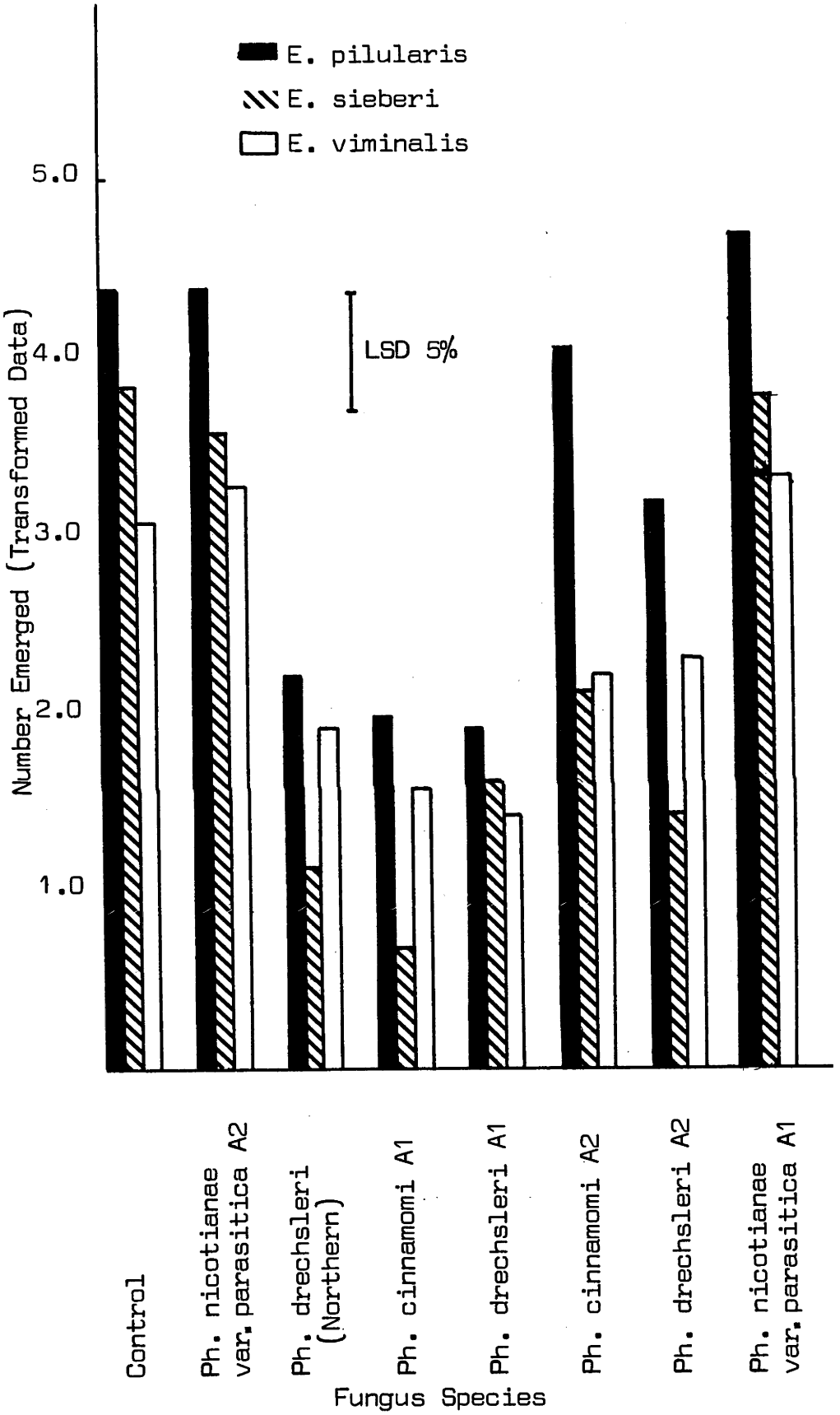

FIG. 4.3 Number of Seedlings Emerged. Fungi x Tree Interaction

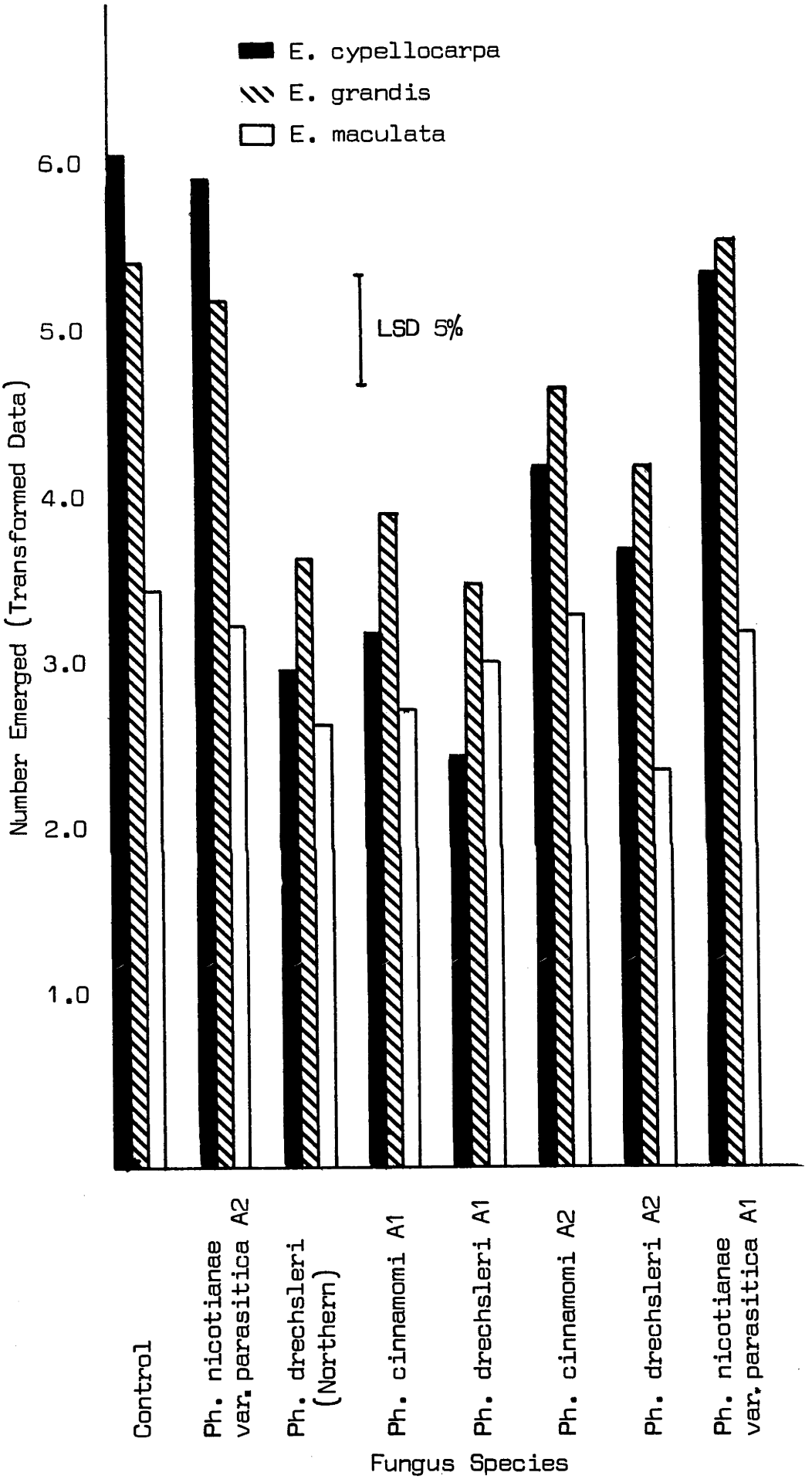

FIG. 4.4 Number of Seedlings Emerged. Fungi x Tree Interaction

TABLE 4.5 Within Tree Species Comparison of the Effects of a Range of Fungal Species on Seedling Emergence. Comparisons Made Using the LSD 5% Shown for the Fungi x Tree Interaction Fig. 4.3 and 4.4

Fungal Species	*Ph. nicotianae* var. *parasitica* A2	*Ph. drechsleri* (Northern)	*Ph. cinnamomi* A1	*Ph. drechsleri* A1	*Ph. cinnamomi* A2	*Ph. drechsleri* A2	*Ph. nicotianae* var. *parasitica* A1
Tree Species							
E. pilularis	N	*	*	*	N	*	N
E. sieberi	N	*	*	*	*	*	N
E. viminalis	N	*	*	*	*	*	N
E. cypellocarpa	N	*	*	*	*	*	N
E. grandis	N	*	*	*	*	*	N
E. maculata	N	*	*	N	N	*	N

N = Not significant at the 5% level.

* = For tree species, reduction in seedling emergence when compared to the control is significant at the 5% level.

The LSD 5% plotted in Fig. 4.6 was used to compare disease caused by various fungi with the controls, for a particular soil. The results of these comparisons are summarized in Table 4.7.

With the exception of the two Ph. nicotianae var. parasitica isolates, all introduced Phytophthora spp. significantly increased the severity of post-emergence damping-off in the Tallaganda soil. In the other soils, fewer Phytophthora spp. caused significant loss when compared with the controls.

All Eucalyptus spp. employed were susceptible to some degree to pre- and post-emergence damping-off by some fungi. Eucalyptus maculata was the species least affected by the fungi used.

Some of the organisms used to inoculate the soils were recovered using the lupin baiting technique of Pratt and Heather (1972).

4.3.2 Experiment 2.

The main effects (Soil Extract and Fungi) and the interaction (Soil Extract x Fungi) are highly significant (Table 4.8). Appendix 5 details the mean class of sporangial formation for each replicate, of each fungus, in each of the soil extracts.

Soil extract has a differential effect on sporangial formation by Phytophthora spp. The number of sporangia formed varied between fungi for a given soil extract.

For all fungi, the number of sporangia was greater in the soil extract from the Wonboyn rain forest soil and least in the extract from the Tallaganda soil. Sporangial numbers were particularly small with isolates of Ph. cinnamomi (A1 and A2) and Ph. drechsleri (A1) in the Tallaganda soil extract.

TABLE 4.6 Analysis of Variance for Proportion of Seedlings Killed (Post-Emergence Damping-Off)

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Soil	2	0.895	48.72 ***
Fungi	7	0.248	13.48 ***
Tree	5	0.794	43.20 ***
Soil x Fungi	14	0.046	2.50 **
Soil x Tree	10	0.109	5.90 ***
Fungi x Tree	13	0.028	1.55 N.S.
Soil x Fungi x Tree	70	0.019	1.05 N.S.
Reference	144	0.018	

*** Significant at the 0.1% level.

** Significant at the 1% level.

NS Not significant.

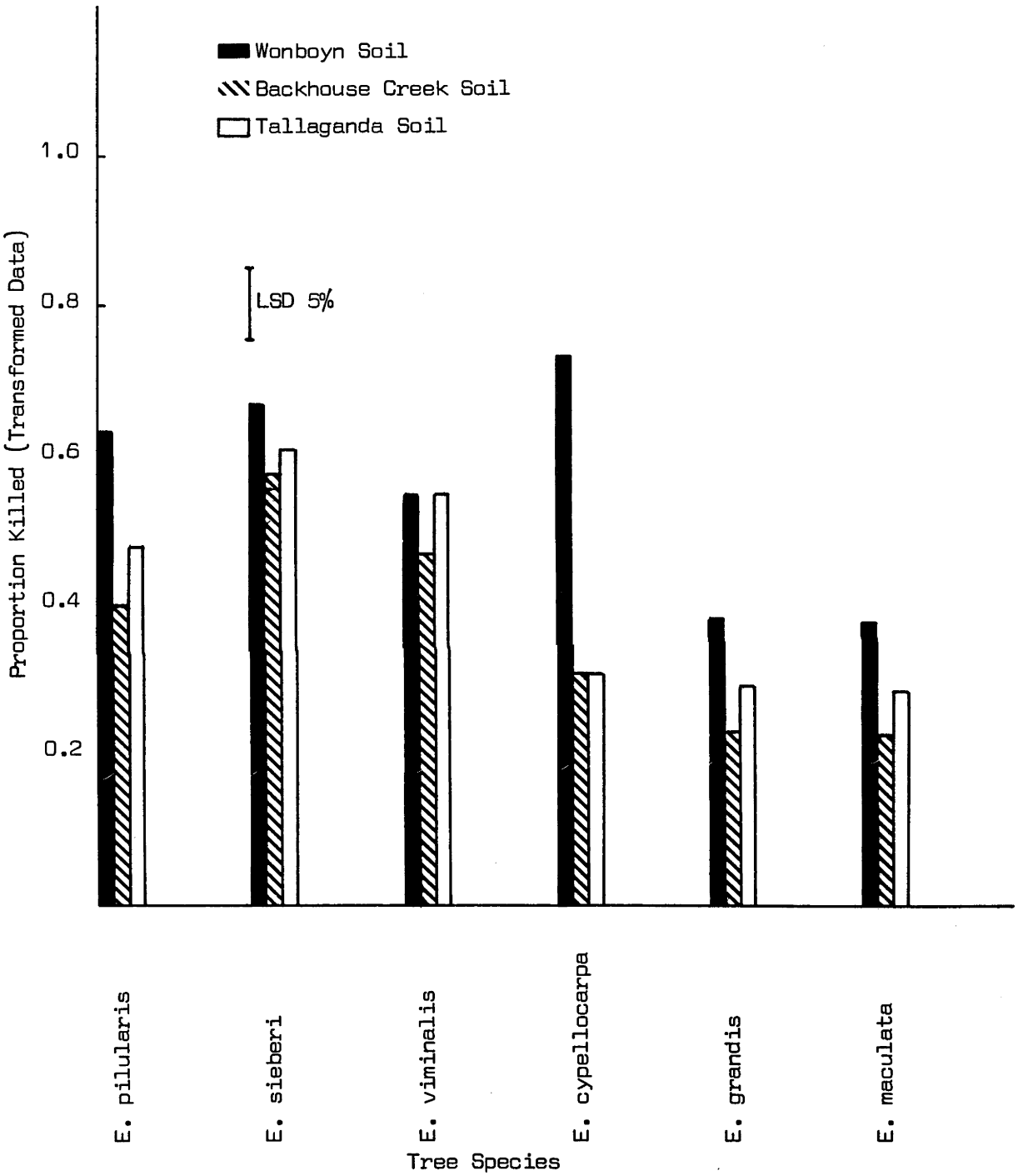

FIG. 4.5 Proportion of Seedlings Killed. Soil x Tree Interaction

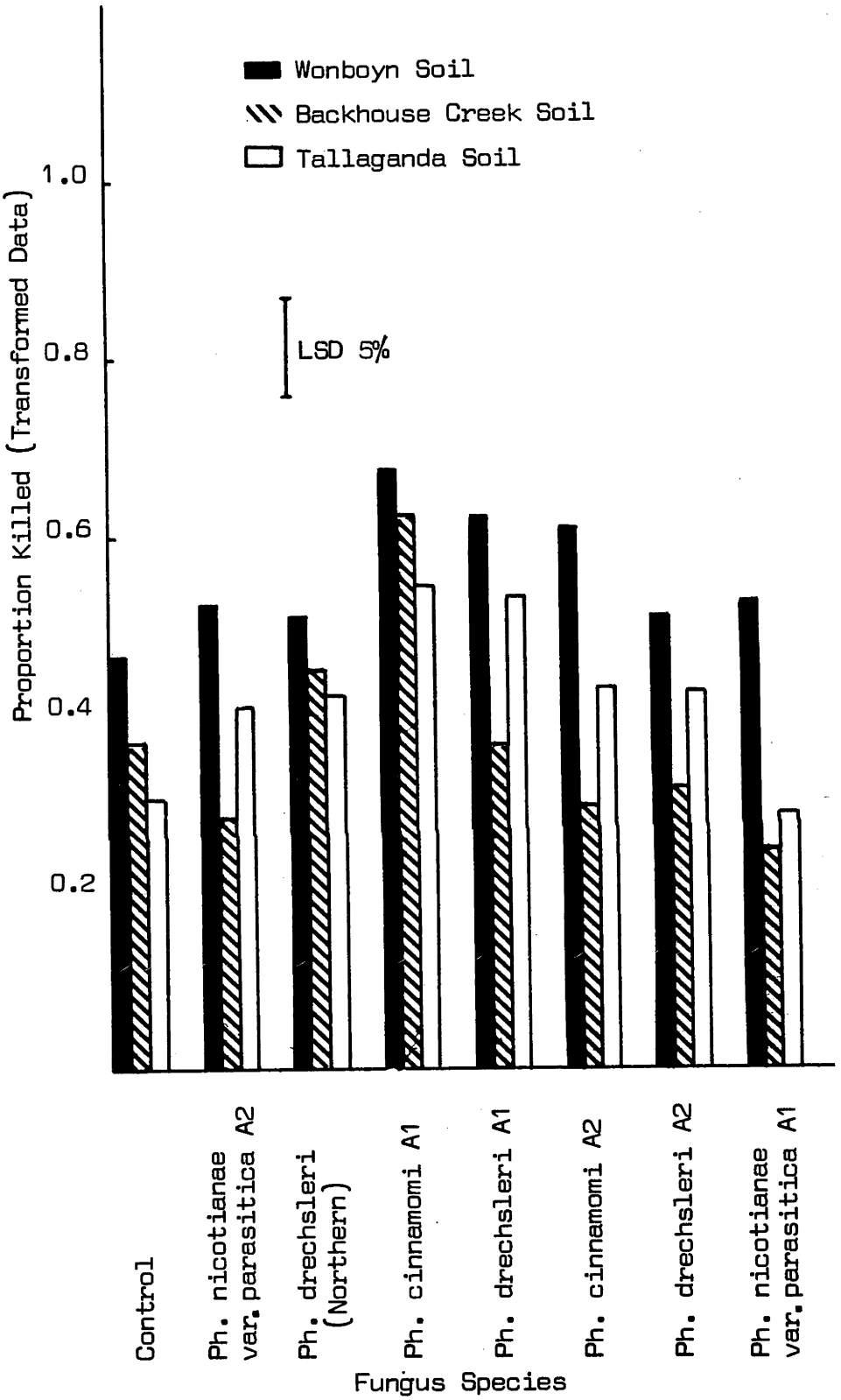

FIG. 4.6 Proportion of Seedlings Killed. Soil x Fungi Interaction

TABLE 4.7 LSD 5% was Used to Compare, Within a Soil, the Effect of Introduced Fungi on the Proportion of Seedlings Killed (Post-Emergence Damping-Off). This Table is a Summary of the Soil x Fungi Interaction Fig. 4.6

Fungal Species	Soil		
	Wonboyn	Backhouse Ck.	Tallaganda
Ph. nicotianae var. *parasitica* A2	N	N	N
Ph. drechsleri (Northern)	N	*	*
Ph. cinnamomi A1	*	N	*
Ph. drechsleri A1	*	N	*
Ph. cinnamomi A2	*	N	*
Ph. drechsleri A2	N	N	*
Ph. nicotianae var. *parasitica* A1	N	N	N

N = Not significantly different from the control at the 5% level.

* = Significantly different at the 5% level.

TABLE 4.8 Analysis of Variance for the Effect of Soil Extract on Sporangial Formation

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Fungi	8	5.76	62.18 ***
Soil Extract	2	7.27	78.54 ***
Fungi x Soil Extract	16	0.77	8.35 ***
Error	27	0.09	

*** Significant at the 0.1% level.

CHAPTER 5

GENERAL DISCUSSION

The experiments described in Chapter 2, investigate the interaction between fungi and seedlings when the biological factors are reduced to a minimum. The introduced fungus faces few if any antagonists and the host grows in a soil environment relatively free from other stimulatory or inhibiting micro-organisms. The introduced fungus is thus likely to then cause the maximum disease of which it is capable, within the physical and chemical limits imposed by the system.

In these experiments, species of *Phytophthora*, *Pythium* and *Fusarium* differed in their capacity to cause disease (Plates 1 and 2). Isolates of *Ph. cinnamomi*, *Ph. citricola*, *Ph. cryptogea*, *Ph. drechsleri* and *Ph. nicotianae* var. *parasitica* caused extensive pre- and post-emergence damping-off in most *Eucalyptus* and *Pinus* spp. tested.

P. ?acanthicum, *P. ?acanthophoron*, *P.?deliense*, *P. irregulare*, *P. myriotylum*, *P. splendens* and several unknown *Pythium* spp. caused severe pre-emergence damping-off in most *Eucalyptus* spp., but only a few of these species caused pre-emergence damping-off in *Pinus* spp. (Table 2.5). Their ability to cause post-emergence damping-off was variable and an indication of general trends would oversimplify the situation. Consideration of individual host-fungus interactions is necessary (Table 2.7).

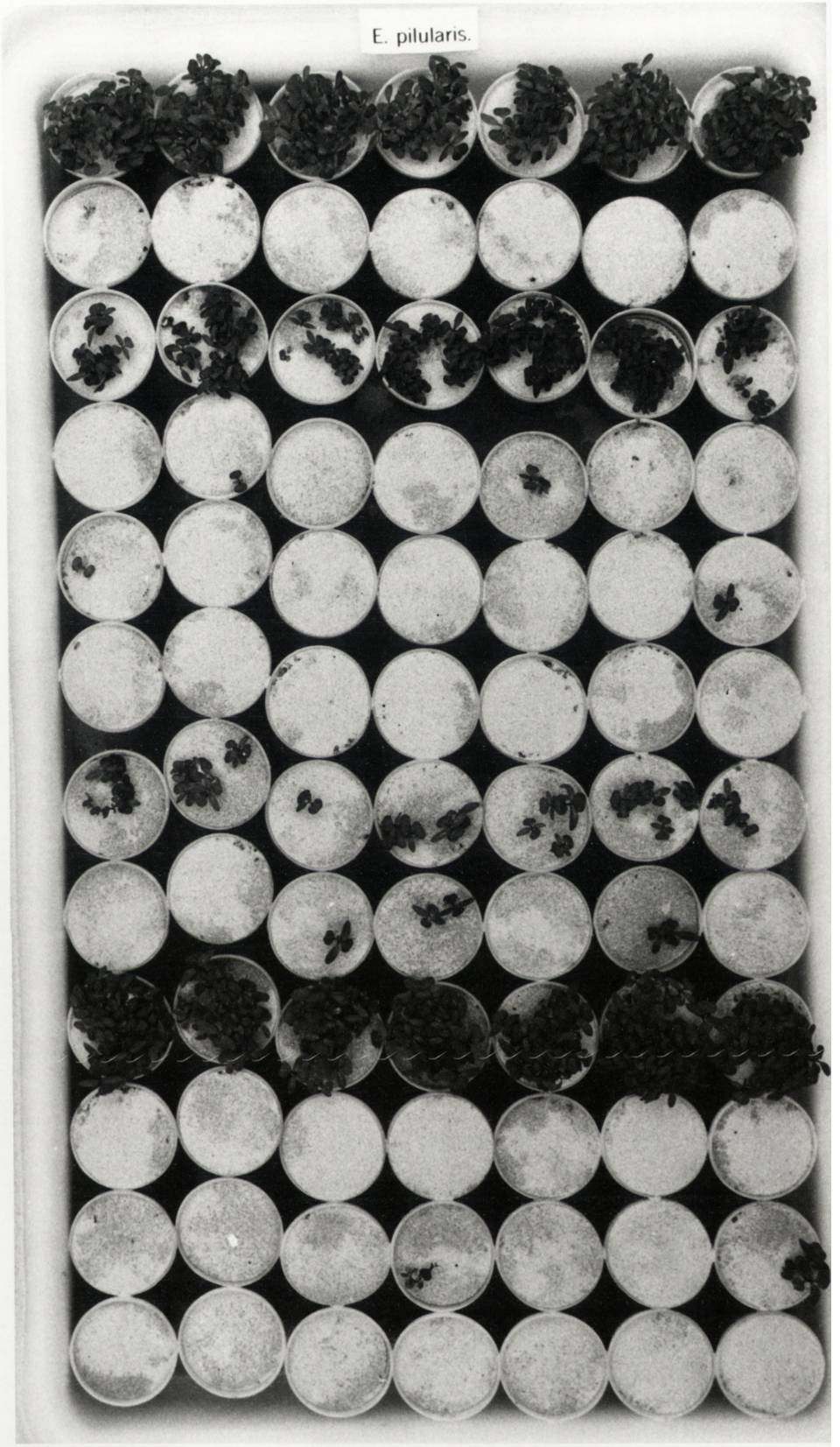

PLATE 1 Pathogenicity of a range of soil-borne pathogens on E. pilularis grown in steam-air-treated sand. This species is classified as being Susceptible to the pathogen Ph. cinnamomi (Pratt & Heather, 1973a).

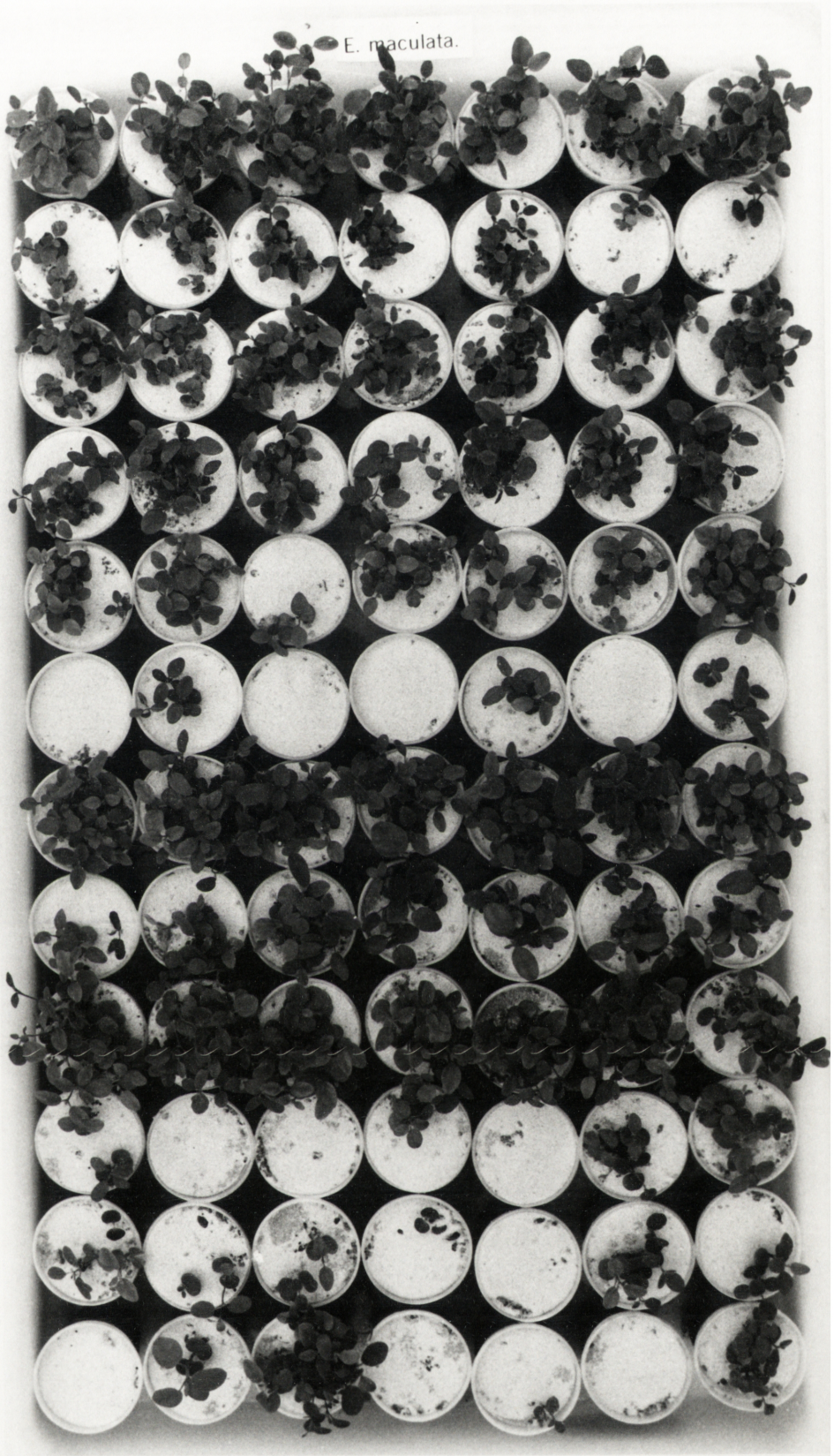

PLATE 2 Pathogenicity of a range of soil-borne pathogens on E. maculata grown in steam-air-treated sand. This species is classified as being Resistant to the pathogen Ph.cinnamomi (Pratt & Heather, 1973a).

The data indicate that fungi causing pre-emergence damping-off do not necessarily cause post-emergence damping-off (Tables 2.5 and 2.7). Such a conclusion may, however, be erroneous as data for post-emergence damping-off are limited (Table 2.7), many organisms having completely prevented the emergence of some host species.

Hosts differed, in general and to specific fungi, in their susceptibility to fungal attack. Both Eucalyptus and Pinus spp. were more susceptible to attack by Phytophthora spp. than to either Pythium or Fusarium spp. All hosts were susceptible to some degree to some fungi but Eucalyptus maculata was outstanding in its overall resistance. Pratt and Heather (1973a) have classified Eucalyptus spp. into four classes according to their field resistance to the disease associated with Ph. cinnamomi:

Highly Susceptible: E. marginata, E. regnans, E. sieberi

Susceptible: E. pilularis

Tolerant: E. viminalis

Resistant: E. cypellocarpa, E. grandis, E. maculata

The results now presented (Tables 2.5 and 2.7) for other fungi indicate that the pre- and post-emergence damping-off responses are variable so that a general classification of susceptibility to a range of fungi would have a limited value.

Further, under one set of conditions a fungus may be more pathogenic than under others, the test conditions may be optimal for one organism and not another. Temperature has a differential effect on disease severity (Thomson, Athow and Laviolette, 1971). It has also been shown that pre-emergence infection is most severe

at temperatures that are relatively less favourable to the host than to the pathogen (Leach, 1947). There are other factors that are equally important. Griffin (1958) has shown that the change of incidence of damping-off with change in soil pH is strongly negatively correlated with change in growth rate of the host.

Where pathogenesis has not been shown, an additional organism may be required to initiate suitable infection conditions. Such synergism in the natural system may be more important than has been indicated in the past. Synergism occurs within fungal genera (Pythium complexes, (Hendrix and Campbell, 1970)), between genera (Pythium-Fusarium complexes of agricultural crops (Kerr, 1963; Pratt, 1965; Frank, 1972)), and between nematodes and fungi (Ruehle, 1973).

Different isolates of the same fungal species varied in their pathogenic spectrum. Ph. drechsleri (Northern, A1 and K93 isolates) caused post-emergence loss in Eucalyptus maculata, but isolates (J8 and J15) did not cause a similar loss (Table 2.7). Examination of this table and Table 2.5 provides further similar instances. Some fungi tested here and found not to be pathogenic, have been shown by other workers to be pathogenic under similar test conditions. Thus, Oxenham and Winks (1963b) have shown that P. ultimum was highly pathogenic on Pinus radiata, causing extensive post-emergence losses and that P. splendens was pathogenic on Pinus elliottii in their experiments. The literature contains reports of F. oxysporum being pathogenic on some species and not on others, there are also reports of variation in the degree of pathogenicity between isolates of Fusarium spp. (Oxenham and Winks, 1963b; Edmonds and Heather, 1973).

Chapter 3 introduces a new variable, the microbial population existing in each of the two soils. The natural soil microflora, in conjunction with the physical and chemical components of the soil, may affect the ability of introduced pathogens to cause damping-off of seedlings (Oxenham and Winks, 1963b; Vaartaja, 1967). In these experiments, nearly all fungi demonstrated similar abilities to cause pre- and post-emergence damping-off to those established in the experiments described in Chapter 2 (Plates 3 and 4). Ph. nicotianae var. parasitica was the only exception, little damping-off occurring in either of the non-sterile soils.

The plants grown in the soil normally supporting Eucalyptus cypellocarpa were larger than those grown in the soil normally supporting Eucalyptus sieberi (Plates 3 and 4). This can be attributed to the higher nitrogen and phosphorus content of the former soil (Section 3.2.1).

Chapter 4 introduces an additional variable to those already discussed, the occurrence of a Phytophthora sp. as a component of the natural soil microflora in two of the three soils.

As in the experiments described in Chapter 3, Ph. nicotianae var. parasitica caused little disease. Pre-emergence losses caused by other Phytophthora spp. were similar in the Wonboyn, Backhouse Creek and Tallaganda soils (Table 4.3). No consistent pattern of post-emergence damping-off occurred that would indicate that the prior presence of Phytophthora spp. had affected directly or indirectly the pathogenicity of introduced isolates.

PLATE 3 Pathogenicity of a range of soil-borne pathogens on E. cypellocarpa grown in soil normally supporting E. cypellocarpa.

PLATE 4 Pathogenicity of a range of soil-borne pathogens on E. cypellocarpa grown in soil normally supporting E. sieberi.

Disease symptoms have been produced on many of the hosts under each of the three experimental conditions used. Reproduction of disease symptoms is inadequate on its own, the causal organism must also be isolated. Isolation of the organism used in the original inoculation could be achieved readily from the roots of dying seedlings, under the experimental conditions described in Chapter 2. However, by including either the natural microflora (Chapter 3) or a Phytophthora sp. and natural microflora (Chapter 4) as an additional variable in the test system the ease with which the fungus used in the original inoculation could be recovered, was decreased. In this latter case the natural pathogenic component or the introduced pathogen could be recovered from the roots of only Eucalyptus pilularis, Eucalyptus sieberi and Eucalyptus viminalis seedlings. It was more difficult to isolate soil-borne pathogens from the roots of plants classified as being field resistant by Pratt and Heather (1973a).

The ability of individual soils to induce sporangial formation and hence dissemination of the pathogen is possibly, a significant factor in determining disease severity. In the experiment described in Section 4.3.1.2, sporangial formation was studied in various non-sterile aqueous soil extracts. The ability of the soils to induce sporangial formation was variable (Appendix 4), isolates of Ph. cinnamomi and Ph. drechsleri (A1) producing few sporangia in the Tallaganda extract. The difference may be attributable to the different microbial population of the soils, as soil micro-organisms have been implicated in inducing sporangial formation (Marx and Haasis, 1965; Chee and Newhook, 1966; Ayers and Zentmyer, 1971).

In this project a number of other Phytophthora and Pythium spp. in addition to Ph. cinnamomi were shown to be pathogenic, causing pre- and post-emergence damping-off in many of the Eucalyptus and Pinus spp. used (Tables 2.5 and 2.7). Ph. drechsleri is frequently recovered from forest soils (Pratt and Heather, 1973b) and some isolates could be rated as being comparable in pathogenicity with Ph. cinnamomi. Some of the other organisms tested were also of comparable pathogenicity, but their rare isolation from forest soils suggests that they may not be important in determining the vegetational pattern of native communities.

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APPENDIX 1 PATHOGENICITY TRIALS

Fungal Species		Control							Fusarium moniliforme var subglutinans							Fusarium oxysporum						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	26	22	22	12	20	3	30	13	19	22	16	9	9	10	12	19	11	17	18	16	15
	b	26	22	22	12	30	3	30	14	19	25	18	12	12	17	13	23	12	17	18	16	17
E. sieberi	a	18	22	18	33	30	10	10	5	26	15	24	25	22	17	21	24	11	7	6	15	10
	b	18	22	18	33	30	10	10	6	26	15	24	25	22	17	21	25	13	7	6	17	10
E. marginata	a	3	0	4	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	3	0	4	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. regnans	a	0	7	2	6	8	4	4	1	2	0	2	3	0	3	1	2	4	0	1	0	2
	b	0	7	2	6	8	4	4	1	2	0	2	3	0	4	1	2	4	0	1	0	2
E. viminalis	a	16	16	16	12	10	14	14	4	6	2	3	6	4	4	19	8	10	7	23	5	25
	b	16	16	16	12	10	14	14	4	6	2	3	6	4	4	19	8	10	8	23	5	26
E. cypellocarpa	a	39	48	43	34	15	23	36	14	35	25	31	36	8	31	45	25	40	33	32	31	31
	b	39	48	43	34	15	23	36	28	35	28	31	36	32	37	57	27	43	33	32	31	33
E. grandis	a	31	37	42	40	53	35	42	19	18	3	11	18	28	10	53	30	37	29	30	21	20
	b	31	37	42	40	53	35	42	19	18	6	12	18	28	11	53	30	37	30	30	21	20
E. maculata	a	9	9	14	6	13	10	10	9	9	9	15	14	8	16	15	19	15	12	12	12	14
	b	9	9	14	6	13	10	10	9	9	9	16	14	8	17	15	19	15	14	12	13	14
P. radiata	a	9	3	2	2	1	1	1	1	1	2	5	2	2	1	5	0	0	0	1	3	2
	b	0	3	2	2	1	1	1	1	1	2	6	2	2	1	5	1	0	0	1	3	2
P. pinaster	a	0	1	0	1	2	3	0	1	1	1	1	1	1	0	1	2	0	1	1	2	2
	b	0	1	0	1	2	3	0	1	1	1	1	1	1	0	1	2	0	1	1	2	2
P. elliottii	a	12	15	16	15	13	17	14	12	14	11	10	12	14	13	11	13	8	9	4	11	5
	b	12	15	16	15	13	17	14	15	14	11	10	14	14	13	11	13	9	9	5	11	5
P. caribaea	a	7	2	5	4	10	7	9	10	8	8	9	7	7	4	4	2	3	3	4	4	8
	b	7	2	5	4	10	7	9	11	8	9	10	8	7	5	4	3	3	3	6	4	8

a = Seedlings Alive b = Seed Germinated

APPENDIX 1 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Pythium sp. J7							Pythium sp. J9							Pythium myriotylum						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	0	2	0	0	0	0	0	3	1	1	7	2	2	1	0	0	0	0	0	0	0
	b	0	2	0	1	0	0	0	3	1	2	8	2	2	1	1	1	0	1	0	0	0
E. sieberi	a	0	1	0	0	3	1	0	3	0	5	2	1	1	1	0	0	0	0	0	0	0
	b	1	1	0	1	4	2	0	5	1	6	3	1	1	1	0	0	0	0	0	0	0
E. marginata	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. regnans	a	0	1	1	1	1	0	2	2	0	2	2	1	0	0	0	2	2	0	1	0	0
	b	0	1	1	1	1	0	2	2	0	2	2	1	0	0	0	2	2	0	1	0	0
E. viminalis	a	0	4	1	1	2	0	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	5	2	1	3	0	0	2	8	0	0	0	0	0	0	0	0	0	0	0	0
E. cypellocarpa	a	3	0	1	5	0	0	4	0	4	0	4	2	1	2	0	0	0	0	0	0	0
	b	5	0	1	7	0	0	5	2	4	1	5	2	1	2	0	0	0	0	0	0	0
E. grandis	a	3	1	0	5	0	4	0	3	0	2	3	0	0	6	0	0	0	0	0	0	0
	b	4	1	0	5	0	4	0	3	0	3	4	0	0	6	1	0	0	0	0	0	0
E. maculata	a	0	2	10	5	0	1	2	14	19	12	8	8	10	7	0	0	1	0	0	0	0
	b	0	2	10	5	0	2	3	14	20	12	8	8	11	7	0	0	1	0	0	2	0
P. radiata	a	1	1	1	3	1	2	1	2	1	0	1	0	0	3	0	1	0	0	0	0	0
	b	2	1	1	3	1	2	1	2	1	2	1	1	0	3	1	1	2	1	0	1	0
P. pinaster	a	0	1	0	2	0	2	1	3	2	5	0	1	1	3	0	0	1	0	0	0	0
	b	1	1	0	3	0	2	1	3	2	5	0	1	1	3	1	0	2	0	1	0	0
P. elliottii	a	10	8	13	13	13	9	11	14	10	14	11	12	15	16	0	2	1	6	3	0	3
	b	10	8	13	14	13	9	11	15	10	14	12	13	16	16	2	4	4	17	6	6	7
P. caribaea	a	5	6	9	11	2	10	5	9	6	8	8	7	15	7	2	0	0	0	0	0	2
	b	5	6	9	12	2	10	5	10	6	8	8	7	15	7	4	1	1	0	0	1	5

a = Seedlings Alive b = Seed Germinated

APPENDIX 1 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Pythium deliense							Pythium splendens							Phytophthora cryptogea J11						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	0	0	2	3	0	0	0	1	1	0	1	1	0	1	1	2	1	2	1	0	0
	b	0	0	2	3	0	0	1	2	4	0	3	2	0	1	1	2	1	2	1	0	0
E. sieberi	a	0	0	0	0	0	0	1	0	0	0	0	0	0	4	0	0	0	0	0	0	0
	b	0	1	0	0	1	0	2	1	0	0	0	0	0	4	0	0	0	0	0	0	0
E. marginata	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	b	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
E. regnans	a	0	0	0	0	0	0	0	1	0	0	3	1	1	1	1	0	0	1	0	0	0
	b	0	0	0	2	0	0	0	1	0	0	3	1	1	1	1	0	0	1	0	0	0
E. viminalis	a	2	1	2	0	7	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	b	2	1	2	2	7	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
E. cypellocarpa	a	0	6	2	5	0	0	0	0	0	0	0	1	1	3	0	0	0	0	0	0	0
	b	0	9	4	6	0	0	0	2	0	0	0	1	1	6	0	0	0	0	0	0	0
E. grandis	a	0	1	0	0	0	1	1	4	0	13	15	16	5	7	4	1	3	6	4	0	2
	b	1	2	0	0	0	1	1	4	0	14	16	18	6	7	4	2	3	7	4	0	2
E. maculata	a	2	1	1	1	4	3	11	2	2	0	1	0	2	0	1	0	0	0	0	4	0
	b	3	3	2	1	5	3	11	2	2	0	1	0	2	0	1	0	0	0	0	5	0
P. radiata	a	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0
	b	0	0	0	1	1	2	0	1	2	0	1	1	0	0	2	0	0	1	0	0	0
P. pinaster	a	0	0	1	2	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	b	0	0	1	2	1	3	2	0	0	0	2	1	1	0	0	1	0	1	0	1	0
P. elliottii	a	7	4	14	13	8	11	3	7	6	6	9	7	9	11	2	4	2	3	1	0	2
	b	13	7	14	15	10	13	10	7	7	8	11	8	10	11	4	5	5	3	5	4	7
P. caribaea	a	1	2	4	3	2	1	4	0	1	3	3	2	2	4	1	1	1	2	1	0	3
	b	1	6	9	5	3	2	5	1	2	3	5	4	2	11	4	2	5	5	2	1	4

a = Seedlings Alive b = Seed Germinated

APPENDIX 1 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Phytophthora cryptogea J12							Phytophthora drechsleri J8							Phytophthora drechsleri J15						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	0	0	0	0	1	1	0	3	1	0	0	1	0	2	0	0	0	0	0	2	0
	b	0	0	0	0	1	1	0	5	2	1	0	3	1	3	0	1	0	0	2	2	0
E. sieberi	a	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
	b	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	1	3	0	0	0
E. marginata	a	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1
	b	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	0	0	1
E. regnans	a	0	0	0	0	0	0	1	0	0	1	2	0	0	1	2	0	4	1	1	1	1
	b	0	0	0	0	0	0	1	0	0	1	2	0	0	1	2	0	4	1	1	1	1
E. viminalis	a	0	0	0	0	0	0	0	0	2	0	0	0	1	3	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	1	6	1	0	0	3	4	0	0	0	0	0	0	0
E. cypellocarpa	a	0	0	0	0	0	0	0	4	3	2	0	0	2	1	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	9	10	6	2	0	5	2	0	0	0	0	0	0	0
E. grandis	a	1	4	0	0	1	5	2	1	3	1	1	0	0	4	2	0	0	3	0	1	1
	b	1	4	0	0	1	5	2	2	4	4	2	0	0	4	2	0	0	3	0	1	1
E. maculata	a	0	0	0	2	0	0	0	0	0	0	0	1	1	0	2	1	0	2	1	5	7
	b	0	2	0	2	0	0	0	0	1	0	0	1	1	0	2	1	1	3	1	6	8
P. radiata	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	0	0	0	1	0	0	0	1	2	1	0	1	0	0	0	0	4	1
P. pinaster	a	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	1	0	1	0	0	0
	b	0	0	0	0	0	1	1	0	0	1	0	0	0	0	2	1	0	1	0	0	0
P. elliottii	a	1	1	0	10	0	4	1	4	1	2	3	3	2	3	4	1	5	1	2	4	7
	b	3	4	0	12	4	9	8	4	1	2	6	5	6	3	6	6	10	2	3	6	10
P. caribaea	a	0	0	0	0	0	1	0	1	1	1	2	0	0	1	0	0	2	0	2	0	0
	b	3	2	0	1	0	1	0	2	3	4	4	2	0	2	4	1	2	2	2	1	5

a = Seedlings Alive b = Seed Germinated

APPENDIX 2 PATHOGENICITY TRIALS

Fungal Species		Control							Rhizopus oligosporus						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species															
E. pilularis	a	32	26	24	22	20	30	31	26	28	30	25	22	32	24
	b	32	26	24	23	21	30	31	26	28	30	25	22	32	24
E. sieberi	a	28	31	39	25	31	32	19	28	30	30	21	20	35	33
	b	28	31	39	25	31	32	22	28	30	30	21	28	35	33
E. marginata	a	11	15	7	11	14	7	10	10	13	16	11	13	9	10
	b	11	15	8	11	14	9	10	10	13	16	11	13	9	10
E. regnans	a	5	5	8	5	8	10	8	1	4	9	6	4	10	3
	b	5	5	8	5	8	10	8	1	4	9	6	4	10	3
E. viminalis	a	17	14	15	18	10	12	13	16	19	14	14	13	15	15
	b	17	17	15	18	10	13	13	16	19	14	14	13	15	15
E. cypellocarpa	a	48	56	44	30	25	39	42	41	52	39	46	35	49	31
	b	48	56	44	30	25	39	43	41	52	39	46	35	49	31
E. grandis	a	34	28	27	23	40	13	25	23	22	20	18	27	20	19
	b	34	28	27	23	40	13	25	23	22	20	18	27	20	19
E. maculata	a	12	16	20	11	8	11	14	15	12	12	9	15	19	8
	b	12	16	20	11	8	13	14	15	12	12	10	15	19	8
P. radiata	a	19	20	18	13	11	10	6	19	19	18	19	15	16	18
	b	19	20	18	17	15	16	16	19	19	18	19	15	16	18
P. pinaster	a	10	11	7	11	6	7	9	14	8	7	9	12	11	10
	b	10	11	7	11	6	7	9	14	8	7	9	12	11	10
P. elliottii	a	16	14	15	13	15	10	13	8	11	15	11	13	12	7
	b	16	14	15	13	15	10	13	8	11	15	11	13	12	7
P. caribaea	a	14	9	16	16	5	5	6	9	10	10	12	10	12	10
	b	14	9	16	16	5	5	6	9	10	10	12	10	12	10

a = Seedlings Alive b = Seed Germinated

APPENDIX 2 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Pythium?acanthophoron							Pythium ultimum var. sporangiferum							Pythium?acanthicum						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	3	0	0	1	0	0	1	27	38	23	19	24	21	30	0	0	0	0	0	0	0
	b	4	0	0	5	0	1	2	27	38	23	19	24	21	31	0	0	0	0	0	0	0
E. sieberi	a	0	0	1	2	2	2	2	28	41	22	28	30	37	33	0	0	0	0	0	0	0
	b	0	0	1	3	3	4	6	28	41	22	29	30	39	33	0	0	0	0	0	0	0
E. marginata	a	8	3	4	1	4	8	4	11	14	12	10	13	6	12	0	0	0	0	0	0	0
	b	8	3	5	1	7	9	5	11	14	12	11	13	6	12	0	0	0	0	0	0	0
E. regnans	a	0	0	0	0	0	0	1	9	15	11	12	7	14	8	0	0	0	0	0	0	0
	b	1	0	0	0	1	0	2	9	15	11	12	10	14	8	0	0	0	0	0	0	0
E. viminalis	a	4	3	3	0	0	3	1	7	9	11	3	14	13	8	0	0	0	0	0	0	0
	b	6	3	3	1	2	9	2	7	9	11	3	16	13	8	0	0	0	0	0	0	0
E. cypellocarpa	a	1	5	0	0	0	10	0	37	21	33	41	24	30	38	0	0	0	0	0	0	0
	b	6	10	8	2	5	16	17	37	21	33	41	24	30	38	0	0	0	0	0	0	0
E. grandis	a	1	1	6	2	2	3	8	30	24	14	2	23	13	14	0	0	0	0	0	0	0
	b	1	1	7	2	4	3	12	30	24	14	2	23	13	14	0	0	0	0	0	0	0
E. maculata	a	2	5	0	2	2	2	4	13	16	11	15	18	18	17	7	0	1	0	17	1	0
	b	6	6	0	2	4	3	4	13	16	11	15	18	18	18	8	2	1	0	18	2	1
P. radiata	a	13	19	16	15	9	17	18	20	19	19	17	18	19	18	6	5	15	6	2	3	8
	b	13	19	16	15	9	17	18	20	19	19	17	18	19	18	8	8	15	7	3	4	12
P. pinaster	a	5	9	1	6	7	10	7	9	8	11	8	6	9	11	0	1	5	2	5	4	1
	b	6	9	2	7	7	10	7	9	8	11	8	6	9	11	0	2	6	3	5	4	3
P. elliottii	a	9	13	6	9	7	15	12	12	14	14	11	9	12	11	11	15	6	10	11	11	6
	b	9	13	6	9	7	15	12	12	14	14	11	9	12	11	12	15	6	14	11	11	8
P. caribaea	a	10	15	8	13	10	12	4	9	16	9	12	12	10	11	8	0	6	7	1	0	3
	b	11	15	8	13	10	14	5	10	16	9	13	12	10	11	8	0	6	8	3	0	5

a = Seedlings Alive b = Seed Germinated

APPENDIX 2 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Pythium middletonii							Pythium irregulare							Pythium? oedochilum						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	10	7	15	7	8	17	7	0	0	0	0	0	0	0	8	4	14	10	17	9	11
	b	10	7	15	7	9	17	8	0	0	0	0	0	0	0	10	10	15	12	19	10	14
E. sieberi	a	20	21	11	10	17	13	10	0	0	0	0	0	0	0	5	8	4	9	9	4	8
	b	20	21	11	10	17	13	10	0	0	0	0	0	0	0	5	9	6	11	9	5	9
E. marginata	a	9	7	9	9	11	7	8	0	1	0	0	0	0	0	10	9	6	6	7	7	4
	b	9	8	11	9	11	7	8	0	1	0	0	0	0	0	10	11	6	6	7	7	4
E. regnans	a	5	5	3	6	3	8	1	0	0	0	0	0	0	1	10	2	6	8	8	5	8
	b	5	5	3	6	3	8	1	0	0	0	0	0	0	1	11	4	6	8	8	5	9
E. viminalis	a	14	18	15	15	2	15	9	0	0	0	0	0	0	0	11	18	18	10	17	18	10
	b	14	18	15	15	3	15	9	0	0	0	0	0	0	0	11	19	19	11	17	18	11
E. cypellocarpa	a	30	39	41	37	21	29	39	0	0	0	0	0	0	0	30	60	38	50	28	31	28
	b	30	39	42	37	21	29	39	0	0	0	0	0	0	0	30	60	38	50	29	31	28
E. grandis	a	2	2	18	21	19	10	10	0	0	0	0	0	1	0	13	10	14	18	22	25	19
	b	2	2	18	21	19	10	10	0	0	0	0	0	1	0	13	10	14	18	22	25	19
E. maculata	a	12	9	9	10	14	11	14	4	3	0	2	0	0	2	7	12	8	13	9	10	12
	b	13	9	9	10	14	11	14	4	4	0	2	0	0	2	7	12	8	13	9	10	12
P. radiata	a	17	18	18	20	19	20	17	2	0	2	0	1	5	0	18	18	19	19	18	18	20
	b	17	18	18	20	19	20	17	4	0	3	2	1	5	0	18	18	19	19	18	18	20
P. pinaster	a	8	5	10	6	6	6	9	0	0	0	0	0	0	1	9	13	13	9	13	13	6
	b	8	5	10	6	6	6	10	1	0	1	0	1	0	1	9	13	13	9	13	13	7
P. elliottii	a	17	8	16	11	12	13	13	10	8	5	10	6	14	8	10	14	11	10	5	12	13
	b	17	8	16	11	12	13	13	10	9	6	10	6	14	8	10	14	11	10	5	12	14
P. caribaea	a	13	9	7	9	1	7	11	0	4	9	4	2	0	0	12	10	12	11	11	6	16
	b	13	9	8	9	1	7	11	0	4	9	7	4	2	0	12	10	12	11	11	6	16

a = Seedlings Alive b = Seed Germinated

APPENDIX 2 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Phytophthora drechsleri (Northern)							Phytophthora drechsleri A1							Phytophthora drechsleri (Southern) A2						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
	b	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
E. sieberi	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. marginata	a	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
E. regnans	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. viminalis	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. cypellocarpa	a	0	0	4	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	4	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
E. grandis	a	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. maculata	a	9	7	3	8	5	5	12	9	11	8	4	5	8	7	3	0	2	1	0	3	0
	b	9	7	3	8	5	5	12	9	11	8	4	5	9	7	3	0	2	1	0	3	0
P. radiata	a	0	0	0	0	0	0	0	2	1	4	3	3	2	3	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	3	1	4	3	4	2	3	0	0	0	0	0	0	0
P. pinaster	a	0	0	0	0	0	0	0	0	1	3	0	0	1	1	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	2	3	1	3	2	2	0	0	0	0	0	0	0
P. elliottii	a	1	1	1	1	0	1	3	8	9	1	5	6	4	3	0	2	0	0	4	4	2
	b	1	1	1	1	0	2	3	8	10	2	5	7	4	3	0	2	1	1	4	4	3
P. caribaea	a	1	0	0	0	0	2	0	3	2	2	2	1	3	2	0	0	0	0	0	0	0
	b	1	1	3	0	0	2	2	3	3	3	2	2	4	2	0	0	0	0	0	0	0

a = Seedlings Alive b = Seed Germinated

APPENDIX 2 (Con't) PATHOGENICITY TRIALS

Fungal Species		Phytophthora cinnamomi A1							Phytophthora cinnamomi A2							Phytophthora citricola						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	1	0	0	0	0	0	0	10	25	14	27	23	24	11	0	2	0	1	2	0	0
	b	1	0	0	1	0	0	0	12	26	16	31	23	24	11	0	2	0	1	2	0	0
E. sieberi	a	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
E. marginata	a	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	2	3	1	0	2	0	1	0	0	0	0	0	0	0
E. regnans	a	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
E. viminalis	a	0	0	0	0	0	0	0	1	0	0	0	0	1	3	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	1	0	0	0	0	1	3	0	0	0	0	0	0	0
E. cypellocarpa	a	0	5	0	0	1	0	0	14	23	14	18	12	10	8	0	0	0	0	0	0	0
	b	0	5	0	0	1	0	0	14	23	14	19	14	10	11	0	0	0	0	0	0	0
E. grandis	a	0	1	0	0	1	0	1	7	0	1	1	6	6	1	0	0	0	1	0	0	0
	b	0	1	0	0	1	0	1	7	0	1	1	6	6	1	0	0	1	1	0	0	0
E. maculata	a	3	9	5	5	10	3	1	16	10	17	9	13	12	12	6	6	6	6	9	10	4
	b	3	9	5	5	11	3	1	16	10	17	9	13	12	12	6	6	6	6	10	10	4
P. radiata	a	1	0	0	1	0	0	0	3	2	1	3	2	1	0	0	0	0	0	0	0	0
	b	1	0	0	1	0	0	0	5	5	6	5	5	4	3	0	0	0	1	1	0	0
P. pinaster	a	0	1	0	0	0	1	1	1	2	2	1	1	1	2	0	0	0	1	1	0	0
	b	0	1	0	0	0	1	1	2	2	3	4	4	4	6	1	0	0	1	2	0	0
P. elliottii	a	3	1	3	2	1	0	2	5	9	8	9	8	5	6	3	3	2	0	1	2	1
	b	3	3	3	2	3	0	3	5	10	8	9	8	6	6	4	4	3	1	1	2	1
P. caribaea	a	0	1	0	1	1	0	0	10	5	9	10	9	8	3	0	0	1	1	0	0	1
	b	0	1	1	1	3	0	0	10	7	11	12	12	9	3	0	2	3	1	1	1	2

a = Seedlings Alive b = Seed Germinated

APPENDIX 2 (Cont'd) PATHOGENICITY TRIALS

Fungal Species		Phytophthora nicotianae var. parasitica A2. J18							Phytophthora nicotianae var. parasitica A2. J28							Phytophthora cactorum						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Tree Species																						
E. pilularis	a	4	7	4	5	1	7	7	7	4	4	5	3	5	7	24	26	31	30	16	21	28
	b	11	10	12	7	6	14	14	8	6	6	7	4	8	9	25	28	31	32	18	25	28
E. sieberi	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	7	5	8	7	4
	b	1	1	0	0	0	1	0	0	1	0	0	0	0	0	4	12	11	9	13	8	5
E. marginata	a	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	3	1	3	0	2	7
	b	0	0	0	0	0	0	0	1	0	1	0	1	2	0	6	5	3	4	4	4	9
E. regnans	a	0	0	0	0	0	1	1	0	0	1	0	1	5	0	10	5	2	7	6	2	3
	b	0	0	1	0	1	1	1	0	0	1	0	1	6	0	10	5	2	9	8	3	4
E. viminalis	a	3	2	3	2	6	2	1	3	0	1	2	0	0	1	9	3	9	11	8	6	8
	b	5	2	5	2	6	2	1	3	0	1	2	0	0	1	9	3	11	13	11	6	12
E. cypellocarpa	a	18	26	19	15	24	21	9	17	6	5	10	18	4	13	29	35	39	38	37	34	34
	b	19	29	19	16	25	23	16	18	7	4	12	20	9	13	29	35	39	38	39	34	34
E. grandis	a	6	16	0	21	6	13	7	8	6	13	0	0	10	8	11	16	9	14	20	22	25
	b	6	17	0	21	6	13	7	8	6	15	0	0	10	8	11	16	9	14	20	22	28
E. maculata	a	14	14	18	12	12	16	14	11	7	12	11	11	14	12	12	12	10	13	10	13	14
	b	14	14	18	14	12	16	14	17	7	12	11	11	14	12	12	12	11	13	10	13	14
P. radiata	a	0	0	0	0	0	0	0	0	0	0	0	0	1	0	6	7	4	11	9	14	11
	b	1	2	3	1	1	3	2	1	2	0	1	0	1	0	10	7	6	12	9	14	15
P. pinaster	a	0	0	2	1	1	2	2	2	1	2	1	3	1	0	8	11	4	6	6	9	6
	b	0	1	5	2	1	3	6	3	1	3	2	3	2	3	8	11	4	6	6	9	6
P. elliottii	a	3	3	6	3	13	6	8	7	10	5	4	8	4	3	11	8	10	10	8	7	11
	b	4	4	6	4	13	6	8	8	12	5	4	8	4	3	11	9	11	10	8	7	11
P. caribaea	a	0	4	4	7	13	3	7	4	2	8	3	5	3	3	9	8	11	10	10	5	7
	b	2	5	4	8	13	3	8	5	4	9	5	5	3	3	10	9	2	10	10	5	8

a = Seedlings Alive b = Seed Germinated

APPENDIX 3 PATHOGENICITY TRIALS

Fungal Species		Control							Fusarium moniliforme var. subglutinans							Fusarium oxysporum						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
E. cypellocarpa Soil																						
Tree Species																						
E. pilularis	a	16	17	17	8	19	28	22	31	24	35	29	22	18	16	28	18	26	28	13	18	24
	b	17	17	17	8	19	28	22	31	25	37	30	23	19	17	28	19	26	27	14	19	24
E. sieberi	a	14	29	24	19	22	18	18	20	22	14	21	14	14	29	22	21	21	23	25	14	17
	b	14	30	24	19	22	18	18	21	22	15	21	17	14	29	22	21	21	23	25	15	17
E. regnans	a	3	1	2	2	0	2	3	2	0	1	2	0	1	1	1	1	2	0	0	4	0
	b	3	1	2	2	0	3	3	2	0	1	2	0	2	1	2	1	2	0	0	4	0
E. viminalis	a	12	13	14	11	4	12	13	19	14	8	11	14	14	9	15	8	16	7	12	10	14
	b	12	15	14	11	5	13	13	22	14	9	12	17	16	10	17	9	18	8	13	12	14
E. cypellocarpa	a	44	51	46	44	53	46	44	44	32	39	22	47	28	40	44	32	39	22	47	28	40
	b	44	51	46	44	53	46	44	44	32	39	22	47	28	40	46	32	39	23	48	29	40
E. maculata	a	13	6	15	13	13	12	15	11	11	15	12	12	9	12	15	10	8	9	13	17	14
	b	13	7	16	14	15	12	15	11	11	15	12	12	9	12	15	11	9	11	13	17	15
E. sieberi Soil																						
E. pilularis	a	29	32	35	30	33	25	29	29	27	28	33	34	29	35	20	23	30	20	26	13	34
	b	29	33	36	31	33	25	29	29	27	28	33	34	29	35	20	25	32	20	26	13	35
E. sieberi	a	6	18	16	9	18	22	16	14	20	25	17	17	1	18	20	27	16	22	12	16	22
	b	9	25	22	15	21	24	18	18	23	25	18	17	4	20	23	29	17	26	15	18	22
E. regnans	a	3	2	1	1	2	0	3	2	1	2	7	6	3	6	1	4	1	1	3	1	2
	b	3	3	3	3	3	0	3	3	2	2	7	6	4	6	1	4	1	1	3	2	2
E. viminalis	a	12	17	9	9	12	14	14	8	13	19	4	13	14	11	9	5	19	15	6	11	11
	b	13	18	13	10	13	14	18	16	15	23	11	18	17	13	10	7	19	16	12	12	14
E. cypellocarpa	a	51	61	60	49	50	58	62	37	28	30	33	32	26	26	35	43	61	38	49	36	49
	b	51	61	60	49	50	58	62	37	32	32	34	32	27	26	35	43	61	38	49	36	49
E. maculata	a	14	12	14	14	11	9	11	13	13	11	14	13	13	13	9	13	13	15	12	9	15
	b	14	12	15	14	11	9	11	15	13	11	14	13	13	13	9	14	13	15	12	9	15

a = Seedlings Alive b = Seed Germinated

Fungal Species		Phytophthora cryptogea J12							Phytophthora cinnamomi A1							Phytophthora cinnamomi A2						
Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
E. cypellocarpa Soil																						
Tree Species																						
E. pilularis	a	7	11	5	0	2	5	3	7	3	1	4	4	3	2	5	3	7	10	6	8	3
	b	11	12	11	3	9	8	4	9	7	2	8	10	6	7	6	5	8	10	7	8	5
E. sieberi	a	0	3	1	2	1	2	1	0	0	0	0	0	0	0	2	1	0	5	6	0	2
	b	5	4	2	5	3	2	3	2	5	1	1	1	3	2	2	3	2	6	7	2	4
E. regnans	a	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	2	0	2	0
	b	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	2	0	2	1
E. viminalis	a	0	1	1	1	1	2	1	3	1	5	9	0	2	3	0	0	2	0	1	0	5
	b	1	7	2	3	3	4	2	6	1	5	11	4	3	8	1	0	2	0	2	0	5
E. cypellocarpa	a	2	13	4	6	9	7	5	6	6	5	2	9	6	21	1	2	0	2	1	2	3
	b	5	16	7	8	12	7	6	7	14	7	5	10	9	22	2	3	2	5	5	3	0
E. maculata	a	4	4	2	5	3	3	9	7	10	6	9	11	15	7	15	9	14	14	12	12	17
	b	4	4	2	5	3	3	9	7	10	6	10	11	15	7	15	10	14	14	12	13	17
E. sieberi Soil																						
E. pilularis	a	4	8	2	9	2	4	2	4	2	9	10	9	5	5	5	3	5	4	12	7	6
	b	5	15	4	9	5	6	2	12	9	14	15	10	5	9	5	3	5	4	12	7	7
E. sieberi	a	1	4	10	3	0	1	1	2	1	0	0	0	0	1	0	0	0	0	0	2	0
	b	5	6	13	4	1	2	2	4	4	2	4	2	1	4	0	0	1	0	0	3	1
E. regnans	a	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
	b	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1
E. viminalis	a	2	4	6	8	4	2	0	5	4	7	6	1	1	7	0	0	1	0	2	0	0
	b	5	7	9	9	9	5	4	8	5	8	7	4	2	11	0	0	0	1	0	1	0
E. cypellocarpa	a	7	5	0	2	2	2	1	7	1	1	12	6	5	7	0	3	0	3	5	4	4
	b	8	9	0	2	3	4	2	8	3	2	14	8	7	10	0	4	1	6	5	11	5
E. maculata	a	9	7	10	6	12	3	7	10	13	13	11	13	13	15	13	12	14	16	11	18	14
	b	9	7	10	6	12	3	7	10	13	13	11	13	14	15	13	12	16	16	11	18	14

a = Seedlings Alive b = Seed Germinated

Fungal Species			Phytophthora drechsleri (Northern)							Phytophthora drechsleri A1							Phytophthora drechsleri A2 (Southern)						
Replicate			1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
E. cypellocarpa Soil	Tree Species																						
	E. pilularis	a	2	1	0	0	3	4	3	4	6	0	0	0	1	1	0	4	3	0	1	1	4
		b	2	2	1	2	3	5	3	5	8	1	0	1	1	1	2	5	9	2	1	3	4
	E. sieberi	a	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	2	0	2	0	0
		b	1	0	2	1	0	0	0	0	0	2	0	3	0	1	1	0	3	0	4	1	1
	E. regnans	a	1	1	2	0	1	0	0	0	1	0	0	0	0	0	2	0	0	0	0	1	0
		b	1	1	2	0	1	0	0	0	1	0	0	0	0	0	2	1	0	0	0	1	0
	E. viminalis	a	0	1	0	2	2	1	2	2	0	0	0	0	0	1	0	1	0	0	1	2	2
		b	0	1	1	3	2	1	2	2	0	0	0	0	0	1	0	1	0	0	1	2	2
	E. cypellocarpa	a	4	0	0	2	3	2	1	1	4	3	0	2	1	0	0	3	5	2	3	4	3
		b	7	1	4	5	6	6	1	1	7	3	0	2	1	1	1	5	6	2	4	6	5
	E. maculata	a	13	7	6	11	9	11	12	11	17	10	14	7	9	12	5	5	1	4	7	9	3
		b	13	8	6	11	9	11	12	11	17	11	14	11	9	12	5	6	1	4	7	9	3
E. sieberi Soil	E. pilularis	a	7	8	7	15	3	14	15	12	6	4	5	10	7	6	1	2	3	5	2	4	1
		b	10	9	8	15	7	17	16	12	10	6	5	11	7	8	7	6	5	8	3	5	4
	E. sieberi	a	0	0	1	2	0	0	4	4	0	3	0	1	2	0	1	5	0	1	3	3	2
		b	1	2	1	3	0	3	5	6	4	4	0	3	6	1	3	8	0	4	5	4	4
	E. regnans	a	0	4	0	0	4	0	0	0	0	0	0	2	0	1	0	3	0	0	0	1	2
		b	0	4	1	0	5	0	0	0	0	0	1	4	0	1	0	4	0	0	0	1	2
	E. viminalis	a	2	0	1	1	1	0	5	1	0	1	0	1	8	2	2	3	2	0	2	2	1
		b	2	2	1	2	3	0	5	2	1	0	1	11	3		2	5	5	3	3	2	2
	E. cypellocarpa	a	1	10	1	7	2	7	13	1	26	8	5	26	15	26	1	8	0	0	1	5	0
		b	1	13	4	8	4	10	17	1	26	9	8	30	16	28	8	14	3	3	4	6	2
	E. maculata	a	12	14	16	7	13	13	13	10	13	13	11	13	13	15	8	7	17	14	14	11	12
		b	12	16	17	7	13	13	13	11	14	16	11	13	14	15	8	7	17	14	14	13	12

a = Seedlings Alive b = Seed Germinated

APPENDIX 3 (Cont'd) PATHOGENICITY TRIALS

	Fungal Species		Phytophthora nicotianae var. parasitica							Pythium?acanthicum							Pythium irregulare						
	Replicate		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
E. cypellocarpa Soil	Tree Species																						
	E. pilularis	a	28	34	15	10	19	28	17	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		b	29	35	14	16	19	28	14	0	1	0	0	1	0	2	3	0	0	0	3	0	0
	E. sieberi	a	13	12	11	27	20	20	4	3	0	0	0	0	2	0	0	0	0	0	0	0	0
		b	13	12	12	27	21	21	4	3	0	0	0	0	2	0	0	0	0	0	0	0	0
	E. regnans	a	0	1	0	1	2	0	1	2	1	1	4	0	3	0	0	2	0	0	2	1	1
		b	0	1	0	1	2	0	1	2	1	1	4	0	3	0	0	2	0	0	2	1	1
	E. viminalis	a	13	8	8	8	14	10	10	0	1	0	0	1	0	0	2	0	0	0	0	0	0
		b	13	8	8	8	14	10	10	0	1	0	0	1	0	0	2	0	0	0	0	0	0
	E. cypellocarpa	a	17	30	21	46	30	30	27	0	0	0	1	0	1	0	0	0	0	0	0	0	0
		b	17	30	22	47	30	30	28	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	E. maculata	a	15	10	12	13	7	12	11	17	11	10	9	5	9	14	15	0	16	13	11	15	7
		b	15	10	12	13	7	12	13	17	11	12	11	5	9	14	15	3	16	13	11	15	8
E. sieberi Soil	E. pilularis	a	29	27	28	33	34	39	35	0	0	0	0	0	0	2	0	0	0	0	0	0	0
		b	29	27	28	33	34	39	35	0	0	0	0	0	0	2	0	0	0	0	0	0	0
	E. sieberi	a	14	20	25	17	17	1	18	0	0	0	0	1	0	0	0	0	0	0	0	1	0
		b	18	23	25	18	17	4	20	0	0	0	0	1	0	1	0	0	0	0	0	1	0
	E. regnans	a	2	1	2	7	6	3	6	0	1	0	0	1	0	0	0	0	0	1	3	1	0
		b	3	2	7	6	4	6		0	1	0	1	1	0	0	0	0	0	1	3	2	0
	E. viminalis	a	8	13	19	4	13	14	11	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		b	16	15	23	11	18	17	13	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	E. cypellocarpa	a	37	28	30	33	32	26	26	0	0	0	0	0	0	0	0	0	0	1	0	0	0
		b	37	32	32	34	32	27	26	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	E. maculata	a	13	13	11	14	13	13	13	14	9	16	18	12	16	17	15	16	14	0	14	17	17
		b	15	13	11	14	13	13	13	14	9	16	18	12	16	17	15	16	15	4	15	17	17

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 PATHOGENICITY TRIALS

Fungal Species Control

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	20	10	16	10	12	17	14	32	9	5	18	25	26	13	27	2	24	8
	b	25	13	20	14	16	21	18	36	15	8	24	30	26	14	29	9	24	17
E. sieberi	a	16	4	9	5	8	14	11	11	5	17	14	16	13	17	19	14	7	9
	b	20	9	11	9	13	23	15	16	9	23	14	18	15	17	19	16	10	13
E. viminalis	a	11	6	5	9	15	9	1	1	12	6	6	3	10	10	18	6	9	14
	b	13	7	8	15	15	12	1	5	13	8	6	3	10	10	18	13	10	16
E. cypellocarpa	a	22	21	14	20	18	22	7	29	37	36	39	72	58	53	44	34	42	42
	b	29	24	28	32	28	29	15	33	37	36	39	72	58	53	44	36	50	44
E. grandis	a	30	28	15	42	13	12	43	46	25	47	28	13	33	31	35	31	27	15
	b	30	28	18	42	15	23	43	46	25	47	29	15	33	31	35	35	30	22
E. maculata	a	10	9	14	10	14	4	12	15	6	15	17	12	16	15	12	18	7	10
	b	10	11	14	10	14	4	12	15	8	17	17	12	16	15	12	18	7	11

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species Phytophthora cinnamomi A1

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	2	2	3	1	1	2	7	3	5	5	0	4	0	1	0	1	6	2
	b	3	3	7	5	4	4	10	5	9	7	3	5	0	3	0	3	8	4
E. sieberi	a	1	1	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	1	3	5	4	2	1	0	0	0	0	0	0	0	0	0
E. viminalis	a	3	2	1	6	6	2	1	0	4	1	0	0	2	1	0	1	0	1
	b	5	2	2	9	11	7	2	2	11	1	1	0	2	4	0	1	1	1
E. cypellocarpa	a	2	5	0	2	2	3	12	12	21	7	10	11	6	5	22	5	16	20
	b	3	7	4	8	5	3	12	17	26	9	12	15	12	5	26	5	16	22
E. grandis	a	13	23	6	18	8	17	14	13	2	18	11	14	10	13	25	10	19	25
	b	13	26	9	21	9	18	16	15	5	18	13	15	13	15	26	11	20	25
E. maculata	a	4	3	2	10	5	1	11	8	10	10	10	12	6	3	9	9	6	6
	b	5	5	4	12	7	3	11	10	11	10	10	13	6	3	9	10	8	6

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species Phytophthora cinnamomi A2

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	4	9	1	7	6	14	29	20	19	25	19	26	35	5	16	12	13	5
	b	13	12	7	11	9	14	30	20	23	25	22	31	33	16	17	13	14	11
E. sieberi	a	5	4	4	3	2	0	2	0	1	4	10	5	10	6	2	1	2	1
	b	6	10	4	7	6	3	3	0	1	5	12	6	11	9	2	3	3	3
E. viminalis	a	1	7	4	7	2	8	3	2	6	11	8	6	1	1	4	5	1	1
	b	3	8	4	7	6	10	3	2	6	12	8	7	3	2	6	6	3	1
E. cypellocarpa	a	2	3	6	9	4	9	30	32	21	28	40	24	5	6	0	32	27	30
	b	6	11	9	15	14	22	32	34	21	29	46	26	6	7	0	32	27	30
E. grandis	a	11	12	20	18	22	20	37	29	18	32	22	40	7	19	22	36	6	17
	b	15	15	24	20	24	26	39	19	20	32	22	40	7	21	26	36	8	17
E. maculata	a	7	10	8	9	14	9	12	15	9	14	8	14	10	4	11	13	12	14
	b	8	12	10	9	14	11	12	15	9	14	8	14	11	4	11	13	12	14

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species *Phytophthora drechsleri* (Northern)

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	2	1	4	1	1	3	6	2	6	11	0	5	7	7	5	6	6	8
	b	3	1	5	1	2	5	9	4	7	11	0	5	7	11	10	7	6	8
E. sieberi	a	1	3	0	2	2	2	0	0	1	5	0	0	2	2	1	0	2	5
	b	1	3	0	2	3	2	0	0	1	7	0	0	6	3	3	0	2	5
E. viminalis	a	3	4	1	2	1	3	1	2	5	1	11	9	2	3	2	5	1	5
	b	3	4	1	3	1	3	3	2	9	1	11	9	6	5	3	5	1	6
E. cypellocarpa	a	0	2	0	4	3	4	19	17	23	11	14	10	3	8	14	6	12	19
	b	0	5	0	8	3	4	19	22	27	11	15	12	5	9	18	6	12	22
E. grandis	a	3	4	7	1	8	9	3	9	4	30	28	24	7	7	17	20	28	32
	b	3	4	9	4	10	10	6	15	10	30	28	24	17	7	17	20	28	32
E. maculata	a	7	4	2	6	6	7	8	10	12	8	7	8	5	6	10	8	5	7
	b	7	5	2	8	7	8	8	10	12	8	7	8	5	6	10	8	5	7

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species: Phytophthora drechsleri A1

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	4	3	2	4	2	2	7	10	0	2	2	3	2	0	0	1	1	8
	b	5	4	4	6	5	3	11	10	0	3	2	4	4	1	2	2	2	8
E. sieberi	a	3	1	2	2	2	5	3	9	4	1	2	1	2	0	0	1	0	6
	b	6	3	2	4	4	7	5	11	4	1	2	1	3	0	0	2	0	8
E. viminalis	a	2	3	1	0	2	1	9	9	6	0	0	2	1	1	3	4	0	0
	b	5	3	1	1	2	1	9	10	6	0	0	2	2	1	4	4	1	0
E. cypellocarpa	a	0	3	0	1	0	5	15	15	14	3	4	4	1	17	12	5	1	15
	b	0	8	1	6	3	7	15	15	14	3	4	6	2	18	13	5	1	15
E. grandis	a	11	6	2	6	9	9	16	17	18	8	15	12	9	6	8	41	15	14
	b	13	6	3	8	9	9	16	17	18	8	15	16	11	6	9	41	19	15
E. maculata	a	6	11	8	8	6	3	11	8	15	10	12	9	11	5	2	13	9	14
	b	6	12	9	10	9	4	11	8	15	10	12	10	11	5	4	13	9	14

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species Phytophthora drechsleri A2 (Southern)

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	10	2	3	9	6	12	21	19	14	16	14	14	13	5	4	11	1	5
	b	15	2	7	10	8	18	21	19	14	18	14	17	13	5	5	11	1	5
E. sieberi	a	2	2	5	1	2	0	1	1	6	2	2	4	0	0	0	2	0	0
	b	5	3	6	2	2	1	2	2	8	3	3	5	1	0	0	3	2	0
E. viminalis	a	4	4	8	2	8	10	11	7	9	8	5	8	2	0	1	3	1	3
	b	7	6	8	3	11	14	11	7	11	8	5	8	2	0	3	3	1	3
E. cypellocarpa	a	8	2	13	7	14	9	32	19	17	13	10	4	4	15	4	26	17	20
	b	11	2	14	14	19	15	32	19	19	16	16	4	4	15	4	26	18	21
E. grandis	a	11	8	18	16	12	18	34	22	26	18	33	32	10	12	12	14	13	7
	b	17	8	20	19	12	21	34	23	26	18	34	32	10	12	12	16	13	7
E. maculata	a	9	2	4	7	4	9	10	9	11	5	4	8	0	3	4	11	4	7
	b	9	2	4	9	4	9	10	9	11	6	4	8	0	3	4	11	4	8

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species *Phytophthora nicotianae var. parasitica A1*

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	12	10	5	19	16	21	26	25	23	25	18	30	15	20	29	29	27	8
	b	15	17	14	25	18	27	26	25	23	26	18	30	20	23	30	29	27	11
E. sieberi	a	10	7	5	8	16	7	4	13	18	11	5	22	17	11	14	13	16	21
	b	14	10	13	15	19	13	6	15	18	12	7	22	18	11	14	16	18	26
E. viminalis	a	6	7	15	9	12	7	9	11	4	9	10	15	5	3	12	16	6	15
	b	10	13	16	17	14	11	10	12	5	11	11	16	5	4	13	18	6	15
E. cypellocarpa	a	15	12	27	18	13	23	0	35	32	35	29	35	14	24	30	20	46	34
	b	30	23	41	30	33	41	0	38	34	36	32	35	17	24	30	28	52	36
E. grandis	a	30	17	31	19	26	30	35	38	32	39	36	21	25	28	32	26	39	37
	b	30	25	31	22	30	33	35	38	32	39	36	21	25	28	32	26	39	37
E. maculata	a	9	9	7	5	6	16	12	10	14	11	11	12	12	8	5	15	8	12
	b	9	9	7	6	6	16	12	10	14	11	11	12	13	8	8	15	9	14

a = Seedlings Alive b = Seed Germinated

APPENDIX 4 (Cont'd) PATHOGENICITY TRIALS

Fungal Species Phytophthora nicotianae var. parasitica A2

		Wonboyn Soil						Backhouse Creek Soil						Tallaganda Soil					
Plot		A			B			A			B			A			B		
Replicate		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Tree Species																			
E. pilularis	a	11	12	12	6	11	9	25	25	15	29	29	20	9	10	25	22	16	19
	b	19	19	14	11	14	16	27	25	16	32	31	20	9	12	28	24	19	21
E. sieberi	a	7	5	6	8	8	10	13	16	16	13	12	16	12	6	5	13	8	9
	b	10	8	10	16	11	12	15	18	19	15	14	18	13	7	8	14	16	11
E. viminalis	a	11	13	16	9	10	12	9	6	16	10	3	8	5	5	8	8	3	5
	b	14	15	18	11	12	18	9	7	16	15	5	14	8	5	9	9	7	9
E. cypellocarpa	a	18	13	12	29	9	21	53	44	32	29	36	38	40	14	38	48	30	33
	b	34	27	28	47	20	32	54	47	34	29	36	41	43	22	38	48	32	33
E. grandis	a	32	30	13	34	23	20	42	45	35	44	30	28	14	14	6	22	10	11
	b	33	35	16	37	29	21	44	45	35	44	30	28	15	23	13	28	16	14
E. maculata	a	7	10	8	9	12	10	9	12	13	15	14	12	12	6	13	15	4	6
	b	7	11	8	11	13	11	10	13	14	16	14	12	13	6	13	15	4	7

a = Seedlings Alive b = Seed Germinated

APPENDIX 5 MEAN CLASS OF SPORANGIA PER DISC

Fungal Species	Replicate	Soil Extract		
		Wonboyn	Backhouse Creek	Tallaganda
Ph. drechsleri (Northern)	a	4.8	3.8	4.2
	b	4.8	2.8	3.6
Ph. cinnamomi A1	a	3.2	3.2	1.2
	b	3.2	3.4	1.8
Ph. drechsleri A1	a	2.2	1.4	1.0
	b	2.4	1.2	1.4
Ph. cinnamomi A2	a	4.4	4.8	3.2
	b	4.6	4.4	3.6
Ph. drechsleri A2 (Southern)	a	4.2	3.6	3.0
	b	5.0	3.2	3.8
Ph. nicotianae var. *parasitica* A1	a	4.4	5.0	4.4
	b	4.8	5.0	4.0
Ph. nicotianae var. *parasitica* A2	a	5.0	4.4	3.8
	b	5.0	5.0	4.0
Ph. cinnamomi A2*	a	4.4	3.6	1.0
	b	4.4	4.0	1.0
Ph. drechsleri A2**	a	4.0	4.2	3.8
	b	4.0	3.8	3.6

* *Ph. cinnamomi* isolate from the Backhouse Creek Soil
** *Ph. drechsleri* isolate from the Tallaganda Soil

Each replicate is the mean of five discs.

Classification of Sporangia Numbers

1.0	Nil
2.0	Less than 10
3.0	Few
4.0	Medium
5.0	Abundant

APPENDIX 6 MEDIA

1. V8 Juice Medium

20 grams Agar (Oxoid No. 3) concentrate

25 grams V8 Juice (Campbell's V8 Juice concentrate)

1000 mls Glass distilled water

2. Water Streptomycin Agar

20 grams Agar (Oxoid No. 3)

1000 mls Glass distilled water

*$\frac{1}{2}$ ml Streptomycin Sulphate Stock Solution (50ppm)

* Stock Solution: Mix 100 mls of sterile glass distilled water

1 gram Streptomycin Sulphate

3. Eckert and Tsao (1962)

(2ml Pimaricin ("Pimafucin") antibiotic suspension
*(25mg Penicillin (Crystalline Penicillin C)
(25mg Polymixin B Sulphate ("Aerosporin")

8.5gm Cornmeal Agar

500 ml Glass distilled water

* Antibiotics added to cooled Agar